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HUMAN SALIVA

VI. Methods of Biometrical Evaluation of Salivary Analyses[1]

W. W. WAINWRIGHT, D.D.S., M.S. and HERMANN BECKS, Dr. med., Dr. med. dent., D.D.S., F.A.C.D.

The George Williams Hooper Foundation for Medical Research and Division of Dental Medicine, College of Dentistry, University of California, San Francisco, California

INTRODUCTION[2]

In view of the slow progress dentistry has made during the last few decades with regard to some of the outstanding oral problems as caries, paradentosis, root resorption, pathologic bone formation, salivary composition, etc., the need for statistical evaluation of carefully described and classified material has become of paramount importance.

Only few attempts have been made in dentistry to evaluate clinical material properly by biometrical means and many authors who have spent years of valuable time in meticulously recording clinical observations did not avail themselves of scientific means for the interpretation of their findings. Many failed to demonstrate the maximum value of their results because of unfamiliarity with the most effective ways of treating accumulated data. It must be admitted that biometrical procedures require a large amount of time, sometimes even as much as that consumed in accumulating the data themselves.

The fundamental work in biometry of Martin (44), Pearson (47), and Yule (61), has been greatly advanced in recent years by Davenport (9), Fisher (16), Pearl (49), and others with the development of practical methods which can be used without great difficulty in the biological fields.

Biometry (statistical methods specially prepared for the evaluation of biological material) is a science in itself. It is very difficult to master in its entirety. The *application* of the standard biometrical

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methods is not too difficult but may require a new concept of one's problem. To quote Pearl (49):

"The general tendency of all science is to proceed always toward greater and greater precision of results and reasoning. It has elsewhere been pointed out that 'the real purpose of biometry is the general quantification of biology. Its fundamental point of view is that, without a study of the quantitative relations of biologic phenomena in the widest sense, it will never be possible to arrive at a full and adequate knowledge of those phenomena. This point of view insists that a description which says nothing about the magnitude of the thing described is not complete, but, on the contrary, lacks an element of primary importance.' "

For an interested person the terms applied in biometry as well as the many ways of charting are rather bewildering at the beginning, but a study of this subject soon uncovers the many possibilities of treating accumulated data, which provide quantitative methods of: describing a sample or population,[1] idealizing or averaging a population (we often repeat an experiment in order to know what happens on the average), measuring variation and correlation, reducing the bulk of data by expressing the results of extensive observations with a comparatively few numerical values.

For the evaluation and interpretation of numerous salivary analyses in this laboratory it became necessary to make such a study of biometrical methods. The following discussion will deal with the outstanding procedures in biometrical work which are most frequently used and which may form a basis for their application by other investigators.

The use of biometrical methods does not in any sense offer a substitute for careful selection of methods of chemical analysis (or any other technical approach), neither does it describe a sample in the sense of the selection of cases for investigation. The entire plan of a study, including the choice of material, technical approach, and experimental error, must be meticulously considered by each investigator (see (10, 17, 18, 30, 34, 35, 36, 37, 39, 40, 41, 42, 45, 50, 53, 55)). This thoroughness adds far more to the usefulness of an investigation than can any technic of analyzing data. The use of the statistical method is a means of disproving, it never proves that one result is normal and another pathological, but it will state that any given finding is within a certain area of the normal curve, i.e., the frequency of occurrence of such a value. Further, statistical methods enable us to compare

[1] In a statistical sense a result is an "individual," and a group of results is a "population."

groups of values, as given in different publications, in a way which could not be done with a range alone.

In order to simplify the understanding of biometrical procedures, to be used in the establishment of salivary standards, the illustrations in the following discussion have been taken from salivary findings. *All of the values have been arbitrarily chosen for use in the examples and the biological conclusions are not to be interpreted as facts.*

VOLUME OF EXPERIMENTAL MATERIAL REQUIRED FOR BIOMETRICAL EVALUATION AND EXPLANATION OF STATISTICAL TERMS

The first requirement of biometrical evaluation is the selection of a large volume of material. Several hundred cases are desirable because the larger the number (N) of individual investigations, the greater the dependence which can be given the findings and interpretations. Statistical formulas express this statement by saying that successive *large* samples of the same thing ("universe of sampling") will vary less in their characteristics than successive small samples (fifty or under).

For example, the salivary phosphorus content of all living persons would average at a certain value called the "true" average. It is not possible however, to examine saliva on such a large scale, so we must take samples of the population. If possible several investigators should supply results from groups of a few hundred each. The findings from these groups could then be averaged separately. The different averages would not be exactly the same nor would they correspond exactly with the "true" average but they would fall about it within a very narrow range. Formulas are available which express the probable scatter of these group averages about the true one. If instead of *large* samples of several hundred each, the different investigators were to take *small* samples of ten or fifteen, these new averages would not fall within as narrow a range as the larger samples did.

Examples from the literature may explain these general principles of biometry. In their salivary studies, Knoche (32), Krasnow (33), and Entin and Geikin (14), examined varying numbers (N) of patients for phosphorus content as listed in Table I.

The average values (M = arithmetic mean) as calculated by these three authors for their groups (Knoche, 41 cases; Krasnow, 12 cases; Entin and Geikin, 137 cases) vary greatly. All three authors used

accepted biochemical procedures so that the technical phase of the analyses can hardly be made responsible for the differences of the means. There are of course other factors which might influence the mean, as for instance, selective sampling of individuals from limited age groups, etc. The fact that N definitely influences the arithmetic mean, however, can not be denied as demonstrated by the last values of Entin and Geikin (Table I) which were arbitrarily chosen as the last 10 cases from their 137. M in the sample of small N (10) is increased approximately one mg. per cent (from 12.15 to 13.20 per cent), a deviation to which Entin and Geikin, and Krasnow attach great significance in their reports on small samples from studies on pathologic material. The average value (M, arithmetic mean) is usually supple-

TABLE I

The Effect of Large and Small "N" on the Arithmetic Mean (M) of Salivary Phosphorus and the Biometrical Values Derived from N, M and σ

AUTHOR	1 N	2 M P mg. per cent	3 σ_M P mg. per cent	4 σ P mg. per cent	5 σ_σ P mg. per cent	6 V per cent
Knoche	41	17.70	0.99	6.3*	0.49	35.3
Krasnow	12	15.20	0.29*	1.0	0.14*	6.6*
Entin and Geikin	137	12.15	0.16	2.9	0.12	23.6*
	10†	13.20	1.34	4.2	0.67	32.1

* Calculated.

† These 10 cases were taken from the total group of 137 cases (above).

mented by the calculation of the standard deviation (σ) which determines the range or dispersion about M of approximately 68 per cent of all cases (see page 5). The value of the standard deviation is also indirectly influenced by the number of individuals examined in so far as a smaller or larger number of cases far away from the average value will effect σ to a much larger extent if N is small than if N is large.

By decreasing Entin and Geikin's N to 10 cases σ (column 4) is increased almost to that calculated for Knoche's cases which is also based on a small N. Krasnow's cases are not only too small in number, as twelve cases cannot be called a true representative of all "normal" cases, but the standard deviation (σ) shows that the cases were selected and do not represent as wide a range of phosphorus values as

must be expected among normal cases, as observed in the work of other investigators. Knoche does not give the actual records of all of his cases and only presents the results of biometrical interpretation of findings. It is possible to interpret the more than one hundred per cent higher value of the standard deviation (σ) from that of Entin and Geikin (6.3 against 2.78 mg. per cent) as being due in part at least to the difference in size of the samples (41 against 137). The values of columns 3 (σ_M), 5 (σ_s) and 6 (V) are calculated from σ and it is quite evident from Table I that these also vary greatly, due to the difference in size of the samples. These values will be discussed in another section on the significance of differences.

Summarizing, it can be stated that if various authors would analyse approximately the same large number (at least several hundred cases) the standard deviations should vary but little; the same would be true of the arithmetic mean and the reverse of small samples of the same N.

The accompanying explanation of biometrical terms follows generally the works of Hill (24), Pearl (49), Yule (61), and Dunlap and Kurtz (11). The technical procedures for obtaining these values is given in the next section. The great usefulness of Dunlap and Kurtz' standard symbols and glossary of terms is apparent to anyone who studies biometrical texts. Authors rarely use the same symbols. In some texts they have more than one meaning and in others different symbols are used for the same term. Since statistical terms and symbols are not quickly comprehended, an attempt has been made to explain those which are fundamentally important and from which more complicated statistical values of correlations, etc., are calculated.

M is the *arithmetic mean or average* of a series of observations. It may be calculated by summing (Σ) the results ($X_1 + X_2 + X_3 -$) and dividing by the number (N) of observations.

Mdn is the *median* or middle value of all individual results when they are arranged according to magnitude in ascending order. It lies equidistant in rank or order from either extreme (i.e., if there were 51 cases, the Mdn would be the value of the 26th case). It is independent of unusual results at either end of the range, in contrast to M.

After all results of an investigation have been arranged in ascending order and the median (Mdn) established, the *step intervals* (i) are selected, equal in size. The size of the interval depends on the "range" which is the distance from the lowest to the highest value. The longer the distance the larger will be the interval and vice versa. Applied to salivary analysis, the phosphorus values for instance, may lie between 6 and perhaps 50 mg. per cent. The step or class interval should be about 3 mg. per cent. Calcium values are very much closer and therefore the interval should be smaller, about 0.5 mg. per cent for values between 3 and 10 mg. per cent. Both groups give approximately the same number of intervals (15). The number of cases which fall within each interval will vary greatly, depending on the number of recordings.

Arrangement of all values in ascending order and division into equal intervals starting from some whole number, determines the *frequency distribution*. The selection of the step interval for a frequency distribution is very important. All subsequent calculations are made on the assumption that the mid-value of the interval is a true representative (mean) of the cases contained within the interval. It may be found upon a thorough examination of the data that the mean of the cases in several of the intervals do not correspond to the mid-values of the intervals. A classical example of such a condition is taken from the census records. Many people of middle and advanced years are found to give their age on even tens or fives; so that if the step interval values are multiples of five, the means of the intervals would always tend to be lower than the mid-value. The only way to obviate such a condition is to be thoroughly familiar with the original data, both as to content and mode of collection. The establishment of a frequency distribution is most necessary when dealing with a larger amount of material because the combining of individual results into small groups makes it possible for the eye to survey the material and facilitates subsequent calculations (M, σ, etc.).

Mo is the *mode*. It is roughly the *middle* or *mid-value* of the step-interval containing the highest frequency (only if N is large). Mdn and Mo coincide with M if the distribution is symmetrical, i.e., with M at the center of the distribution. However, they will not coincide if M lies to one or the other side of the Mdn, which results in a "skew distribution," characterized by the tendency for the majority of values to be at one or the other end of the distribution.

Mdn and Mo are useful in calculating the degree of skewness. An example of a skew distribution is to be found in statistics on the rate of flow of resting saliva. There are many individuals with rates from 1 to 20 cc./hr (the beginning of the distribution) while there are less with 30, 40, or 50 cc./hr (the middle of the distribution) and still less with 70, 80 or 90 cc./hr. The extreme right or end of the distribution tails off to a very few individuals with high rates of flow of resting saliva, whereas the beginning of the distribution started with a very great number. This is therefore not a symmetrical but a skew distribution.

σ is the *standard deviation*,[4] the most important measure of dispersion of cases around the mean. As stated previously (3, 4, 5, 6, 56, 57) in salivary analytical work, most investigators have given only either the lowest and highest values of their findings or calculated the average or arithmetic mean. In surveying all former reports we find the statement of Hill (24), substantiated "Frequently the worker's analysis comes to an end with this calculation" (meaning the calculation of arithmetic average). The mean and range, however, are not sufficient to give a clear picture of the distribution. Even though this might be facilitated by a calculation of frequency distribution (see above), one needs a measure of variability of each distribution from the mean, in a single figure. This figure may be calculated by adding all negative or positive deviations from the M (ignoring the plus or minus sign) and dividing by the number of observations. The resulting figure would establish the *average or mean deviation* (AD). This measure is seldom used for various reasons and the standard deviation (σ) appears to be more useful in biometrical work because it can be treated algebraically and used to determine correlation and other statistical values which would otherwise not be as easily accomplished.

The standard deviation is obtained by squaring the plus and minus deviations from the mean, dividing by the number of observations, and extracting the square root. From a practical standpoint $M \pm 1\ \sigma$ establishes two values between which approximately 68 per cent of observations will fall. A range of $M \pm 2\ \sigma$ will include approximately 95 per cent of observations and $M \pm 3\ \sigma$ approximately 99 per cent or more of observations.

The values outside of the arithmetic mean $\pm$ 1, 2, or 3 σ represent more or less "rare events" which may be chance findings in the field of biological observations, possibly representing even normal values. On the other hand, they might be expressions of pathologic changes. The evaluation of statistical data especially with regard to biological work rests not alone with the statistician but rather with the clinician who is familiar with the various possibilities of influences on the results. The standard deviation is

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[4] Older terms for the standard deviation are "mean error" (Gauss), "mean square error" and "error of mean square" (Airy). In early usage almost every author had his own designation but the term standard deviation has come into general acceptance.

used to establish a representative range for groups of patients, normals for example, by taking the limits of $M \pm 1\ \sigma$.

V is the *coefficient of variation*. It is used as a measure in place of σ when the spread (dispersion) of two variables are to be compared but which are not measured in the same unit. If applied to salivary analysis we might want to compare the rate of salivary secretion expressed in cc. with salivary content, as for instance inorganic phosphorus expressed in mg. per cent. It is obvious that these two measures (cubic centimeters and miligrams per cent) cannot be compared readily. For this reason the variability of distribution or "standard deviation of the distribution" must be expressed in a different unit, i.e., in percentage. Both percentages can then be compared.

The calculation of the coefficient of variation is therefore:

$$V = \frac{\sigma}{M} \times 100.$$

It is obvious that the larger the proportion of standard deviation to the mean the larger will be the value for "V" and vice versa.

TECHNICAL PROCEDURES OF BIOMETRICAL EVALUATION

In setting up a program for biological investigation of a large amount of material one of the first requirements is that all possible data which are obtainable and which might be useful for biometrical evaluation are properly recorded. How negligent some former investigators were with regard to the description of their patient material, analytical methods employed, age and health of patients has been indicated before (Tables I and III (5) and Table III (6)). The necessity of accurate recordings cannot be emphasized sufficiently and the technical procedure which has been followed in this laboratory for recording the material examined and evaluating it will be the subject of the following discussion.

A. *Method of recording.* In the long process of collection of results from biological material, it is necessary to record the data obtained in a proper manner especially if many hundred individuals are to be examined. If this is not done, considerable difficulties are encountered in subsequent tabulation of the data, resulting in much duplication and time consumed in the transfering and copying of individual values which always carries the hazard of errors.

The routine method of recording the salivary analysis and accompanying examinations of each individual in this laboratory has been arranged in three parts: 1. Master Records. 2. Individual case folders. 3. Detailed salivary analytical records. 1. The master record is the key to all different types of records which are to be preserved. This record chart (Table II), 15 x 22 inches, is adequately ruled for the tabulation of all data pertaining to collection of individual

saliva samples, identity of individuals examined, results of analyses, calculations from original values, and, for a quick survey of all contemplated investigations, the dates of completed examinations (physical examination, nutritional analysis, etc.). This form of master

TABLE II

Master record chart

DATE OF SALIVA EXAM.	CASE NO.	NAME	SEX	AGE	TIME OF COLLECTION			AMOUNT COLLECTED	RATE OF FLOW	Ca	P	Ca	P	Ca × P	Ca/P	Ca × P	→ continued below
					Start	Stop	Min.	cc.	cc./hr.	mg. per cent	mg. per cent	mg./hr.	mg./hr.	mg. per cent	mg. per cent	mg./hr.	
May 16, '35	21	A—, W.	♂	25	8:05	8:35	30	8.8	17.6	7.4	13.8	1.30	2.43	102.1	0.54	3.16	
May 16, '35	22	V—, R.	♂	15	8:10	9:20	70	9.0	7.7	6.7	17.5	0.52	1.35	117.2	0.38	0.70	

CARIES INDEX (BODECKER)				ACIDOPHILUS COUNT	CALCULUS	PARADENTAL CONDITION	→ continued below
Life		Frequency					
Perm.	Decid.	Perm.	Decid.				
41.25	—	3.54	—	20,500	+	—	
29.375	—	1.37	—	60,000	—	—	

EXAMINATION COMPLETED DATE						SUMMARY OF PHYSICAL EXAMINATION
Basal Metabolism	Differential Blood Count	Urinalysis	Nutritional Anal.	Dental Examination	Dental Roentgenograms	
May 18, 1938	May 18, 1938	May 18, 1938	May 18, 1938	May 18, 1938	May 10, 1938	Negative; no metabolic disturb.
May 20, 1938	May 20, 1938	May 20, 1938	May 20, 1938	May 20, 1938	May 15, 1938	Negative; no metabolic disturb.

record also contains the actual results of dental examination (Bodecker's life caries index and caries susceptibility index (7), oral condition, etc.) to facilitate a quick comparison between salivary and dental findings and also for the purpose of finding leads with regard to supple-

mentary branch investigations. Finally a summary is added to indicate whether the investigations accompanying the salivary analysis showed any unusual findings with special regard to metabolic disturbances. 2. The individual case folders contain all accumulated data from the various group investigators for use in future evaluation. Each report is kept separate to facilitate their grouping later on without the necessity of copying them. 3. Since the results of salivary analysis are the outstanding data in this investigation, a special analytical record form is kept for checking of calculations and reference to all original recordings (Table III).

TABLE III

Analytical Record Form

Ca mg. per cent

DATE	CASE NO.	BURETTE READING		CORRECTION SUBTRACTED	CC. 0.01N $KMnO_4$	TITER	RESULT	AVERAGE
		1st	2nd					
4–17–34	1 I	3.12	3.62	0.01	0.49	1.008	4.94	5.0
	1 II	1.00	1.51	0.01	0.50		5.04	
6–8–34	2 I	2.96	3.45	0.01	0.48	1.010	4.85	4.9
	2 II	3.45	3.95	0.01	0.49		4.95	
6–29–34	3 I	0.23	0.58	0.01	0.34	1.000	3.40	3.5
	3 II	0.00	0.37	0.01	0.36		3.60	

B. *Calculation of Mean and Standard Deviation.* The first step in any biometrical evaluation is the computation of the mean and standard deviation. The most elementary method of determining the arithmetic mean consists of adding all individual values and dividing by the number of investigations. If for one reason or another it is the ambition of the investigator only to calculate the arithmetic mean this is the method of choice. However, if the calculation of standard deviation is to be included, as it should, this most time-consuming procedure can be skipped. For this reason a combined procedure would be as follows: Arrange all cases in ascending order,[5] halve the difference from lowest to highest (first and last) result and

[5] Since it is easy enough to pick out of a large number of data the lowest and highest result it is not absolutely necessary to arrange the values in ascending order.

add this figure to the lowest value. This gives the position of the arbitrary mean (M′)[6] which does not correspond to the real arithmetic mean but which leads to it by the use of a correction. For this purpose the deviations (x′) of each individual value from this arbitrary mean are recorded in a second vertical column next to the original data and designated by plus and minus signs. The sum (Σ) of these plus and minus deviations is divided by the number of cases and the resultant figure, on the plus or minus side, is used as the correction of the arbitrary mean giving the actual arithmetic mean (M). This calculation of M is illustrated in Columns 1 to 3 of Table IVA which is self-explanatory. The values of column 3 are again to be used for the calculation of the standard deviation. (See page 11.)

If the accumulated material is large enough to warrant the calculation of a frequency distribution (see next section) a shorter method might be applied.[7] In a collection of calcium determinations from two hundred cases, for instance, the range of 3 to 7 mg. per cent is divided into equal step intervals of 0.5 mg. per cent (column 1, Table IVB) each interval representing a small group of cases. The arbitrary mean is then selected by using the middle value of the center step interval. The center step interval in Table IVB is 4.5 to 4.9 mg. per cent and the midvalue of this is 4.7 mg. per cent. Column 2 indicates how many cases fall within each step interval (frequency distribution). Column 3 gives the deviations from the arbitrary mean by units according to the number of step intervals. Column 4 is the product of columns 2 and 3. In proceeding with the calculation of M the plus and minus values of column 4 are added separately. The resulting two figures subtracted from each other equals plus 118 (+ 178 and −60 = +118). The step value of 0.5 mg. per cent is then multiplied by the result of dividing +118 by 200 (the number of cases). This gives a correction of +0.2950 mg. per cent, which is added to the arbitrary mean of 4.7 mg. per cent finally giving 5.00 mg. per cent, the actual arithmetic mean.

It must be admitted that the procedure seems quite cumbersome, however, if routine methods are established it meets with few difficulties and will reduce the time of calculation considerably in comparison to the two former methods.

[6] If possible the second decimal point should be dropped in order to simplify calculations.
[7] The term "short method" is actually used in biometry.

The calculation of the *standard deviation* (σ) as stated before follows the fundamental formula:

$$= \frac{\text{[Sum } (\Sigma) \text{ of squared deviations from arithmetic mean } (x)^2]}{\text{number } (N) \text{ of cases}}$$

$$\text{or,} \quad = \frac{\Sigma(x)^2}{N}$$

In practice, this procedure is shortened by using the sum of the squares of the deviations from the arbitrary mean (column 4, Table IVA). These have already been calculated (column 3, Table IVA) for the determination of the arithmetic mean by the long method and all that remains is to enter their squares in column 4, Table IVA. The step of squaring removes the negative signs so that all values are now plus. The squares are summed and divided by the number of cases (N = 20). This result must be corrected by subtracting the square (which makes the sign plus in all cases) of the sum of the deviations (column 3 Table IVA). The correction must be made because these deviations were calculated from an "arbitrary mean" (M). The final step requires the extraction of square root, and the standard deviation resulting is 1.06 mg. per cent.

The procedure for "grouped data" (Table IVB) is similar. The products of frequency times deviation (column 4, Table IVB) are again multiplied by the deviation giving frequency times deviation squared in column 5 (Table IVB). The sum of this column ($\Sigma f(x')^2$) is divided by the number of cases (N) and the usual correction (the square of: the sum of the "deviations times frequency" divided by N) subtracted. The square root of this result is multiplied by the step interval value (i) which in this case is 0.5 mg. per cent. The standard deviation for calcium in this distribution of two hundred cases is 0.76 mg. per cent.

C. *Frequency Distribution*. The frequency distribution may be graphically demonstrated for the calcium content of fifty cases in a histogram (61, 49, 24) or bar chart, which shows the number of individuals possessing calcium values within the small groups established for the step intervals, (see page 13). In the illustration given in *fig. 1*, the base or abscissa is marked off in equal intervals (step intervals) of 0.5 mg. per cent; i.e., the number of cases between 3.5 to 3.9 mg. per cent calcium are 1, from 4.0 to 4.4 are 2, etc. The height of the

TABLE IV

Calculation of Mean (M) and Standard Deviation (σ)

A. Long Method (Ungrouped Data)

(1) NO. OF CASES	(2) Ca MG. PER CENT	(3) x' (DEVIATION FROM ARBITRARY MEAN)	(4) $(x')^2$ (DEVIATION SQUARED)
1	3.0	−2.0	4.00
2	3.0	−2.0	4.00
3	3.2	−1.8	3.24
4	3.8	−1.2	1.44
5	4.8	−0.2	0.04
6	4.9	−0.1	0.01
7	4.9	−0.1	0.01
8	4.9	−0.1	0.01
9	5.0	0	0
10	5.0	0	0
11	5.0	0	0
12	5.0	0	0
13	5.2	+0.2	0.04
14	5.3	+0.3	0.09
15	5.6	+0.6	0.36
16	5.7	+0.7	0.49
17	6.0	+1.0	1.00
18	6.2	+1.2	1.44
19	6.6	+1.6	2.56
20	6.9	+1.9	3.61
Σ	100.0	0	22.34

Arbitrary Mean (M'):

$$7.0 - 3.0 = 4.0; \frac{4.0}{2} = 2.0; \mathrm{M'} = 3.0 + 2.0 = 5.0 \text{ mg.\%}$$

Mean: $\mathrm{M} = \mathrm{M'} + \frac{\Sigma x'}{N} = 5.0 + \frac{0}{20} = 5.0 \text{ mg.\%}$

Standard Deviation:

$$\sigma = \sqrt{\frac{\Sigma(x')^2}{N} - \left(\frac{\Sigma x'}{N}\right)^2} = \sqrt{\frac{22.34}{20} - (0)^2} = \sqrt{1.117}$$

$$= 1.06 \text{ mg. per cent}$$

B. Short Method (Grouped Data)

(1) STEP INTERVALS OF Ca MG. PER CENT	(2) f (FREQUENCY)	(3) x' (DEVIATION FROM ARBITRARY MEAN)	(4) fx' (FREQUENCY TIMES DEVIATION)	(6) $f(x')^2$ (FREQUENCY TIMES DEVIATION TIMES DEVIATION)
3.0 to 3.4	3	−3	−9	27
3.5 to 3.9	9	−2	−18	36
4.0 to 4.4	33	−1	−33	33
4.5 to 4.9	67	0	0	0
5.0 to 5.4	36	+1	+36	36
5.5 to 5.9	25	+2	+50	100
6.0 to 6.4	16	+3	+48	124
6.5 to 6.9	11	+4	+44	176
Σ	200		−60 / +178	532

Mean: $\mathrm{M} = \mathrm{M'} + \mathrm{i}\frac{\Sigma fx'}{N}$; $[\Sigma fx' = +178 - 60 = +118]$

$$= 4.7 + 0.5\left(\frac{+118}{200}\right) = 4.7 + 0.5(+0.5900) = 4.7 + 0.2950 = 5.00 \text{ mg.\%}$$

Standard Deviation:

$$\sigma = \mathrm{i}\sqrt{\frac{\Sigma f(x')^2}{N} - \left(\frac{\Sigma fx'}{N}\right)^2} = 0.5\sqrt{\frac{532}{200} - (0.5900)^2} = 0.5\sqrt{2.6600 - 0.3481}$$

$$= 0.5\sqrt{2.3119} = 0.5(1.52) = 0.76 \text{ mg. per cent}$$

column over each step interval indicates the number of cases graphically and the scale for N is given vertically at the left on the ordinate. The position of the mean and the range of M $\pm$ 1 σ are marked off on the calcium scale.

This form is useful for the illustration of many types of variables. For purposes of describing the sample, histograms can be constructed for age distribution, seasonal variation, etc. The preparation of these graphic or visual reports as well as tables and charts from the collected

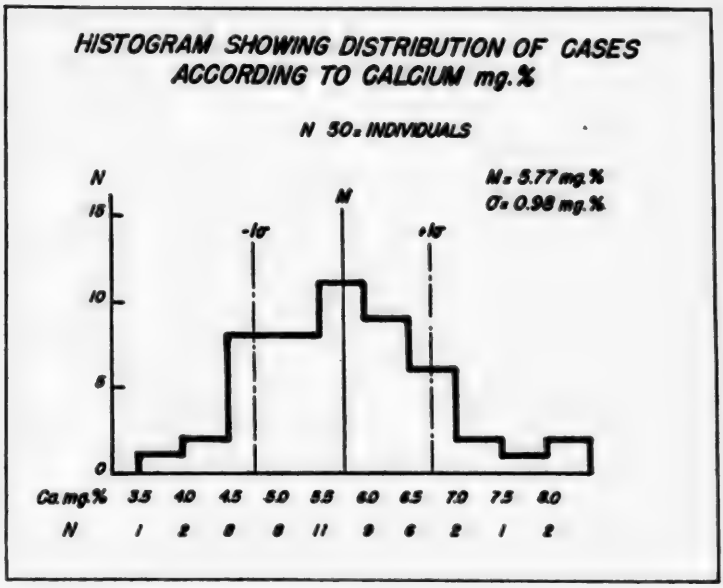


FIG. 1

data depends upon the evaluation under consideration. The desired characteristic or description of the material can be made to "live" for the reader if graphic presentations are thoroughly prepared. These are accomplished by arranging the records, not according to the date of the entry as is the case on the master record, but in most instances by sorting the cases according to age, sex, chemical constituents, etc. Sorting methods will be discussed in a later section. The standard forms for graphs and charts should be followed whenever possible. A

discussion of all graphic methods cannot be elaborated upon here as this is a field in itself, their applications to salivary work, however, will be discussed when the material to be demonstrated calls for a particular type of graph (in this and subsequent publications). The general principles of some graphic methods have been outlined by the Joint Committee on Standards for Graphic Presentation (28) and invaluable

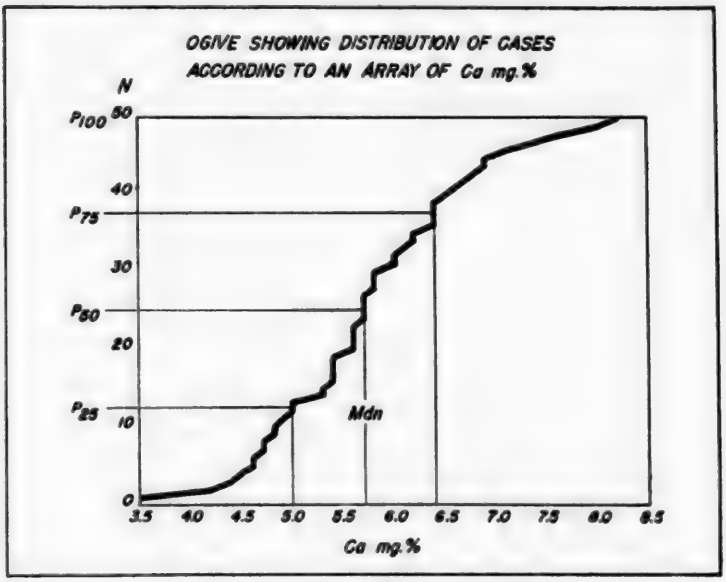


FIG. 2

suggestions are to be found in the works of Karsten (31), Pearl (49), and Yule (61).

Another method of showing the distribution of cases is graphically demonstrated in the ogive of *fig. 2*. It is constructed by plotting all cases on a graph in the order of their magnitude, i.e., in ascending order. The base or abscissa contains for example the scale of calcium values (for the 50 cases of *fig. 1*) while the vertical scale or ordinate shows the rank of the individual values from first (lowest) to last (highest).

The ogive has been used as a means of describing a sample more

frequently in the past in the absence of the methods discussed in this and the previous section. This was accomplished by dividing the vertical scale into: percentiles (P_{25}, P_{50}, etc.; N = 100 per cent, or P_{100}); deciles (equal groups containing 10 per cent of all cases), and quartile deviations (Q) ($M \pm 1Q$ = 50 per cent of cases). The median is readily indicated graphically on an ogive at percentile 50. The ogives of different samples can be plotted on the same scale according to the percentiles instead of the number of cases, permitting the comparison of the different distributions. These methods are now obsolete and are retained only for special applications such as in the field of Education where the scholastic rank of the individuals of a class can be more readily determined than a definite quantitative estimate of their scholastic abilities.

D. *Correlation Coefficient.* The basic tools of biometry are the M, Mdn, and Mo (averages of measures of central tendency), and the AD, (average deviation), σ, and V (measures of dispersion of values about the average). They have been described as to their uses and calculation; of these the M and σ are the most important because they may be used algebraically for the computation of more complicated statistical terms such as measures of correlation. The most useful of the correlation values is the *correlation coefficient*, "r", ("product-moment correlation coefficient"). It was devised by Pearson, (13, 52) to give a measure of the relationship between two variables of a sample. When 50, 60 or more cases are to be evaluated, the calculations are always carried out on a graph.

If in salivary work a possible correlation of age and calcium content is to be investigated (for example in the above 50 cases) the following values have to be calculated for age as well as for calcium: 1. Arbitrary mean (M′). 2. Deviations (x′) from M′ by step intervals taken as units. 3. Frequency of cases in each step interval. 4. Frequency times deviation (fx′) for each step interval. 5. Frequency times deviation squared ($f(x')^2$).

These five values represent fundamental data. The steps of calculation follow those demonstrated in Table IVB, however, in order to be able to more easily follow the steps of the even more complicated formula for the correlation coefficient the resultant values have to be arranged for calcium on the left side of the vertical column, and for

age *below* the horizontal 5 year interval values. Then the frequency distribution of calcium according to age is entered into the corresponding cells within the double lined rectangle of the "correlation grid" (Table V).

The frequency distribution for calcium is the same as in *fig. 1*, with step intervals of 0.5 mg. per cent. The ages are divided into 5 year groups.

To proceed with the working formula for r, as given in Table V, the products of the deviation (x_2') of each cell from the step interval containing the mean of *calcium* times the deviation (x_1') from the step interval containing the mean for *age* must be computed on the basis of the step interval value as a unit. The deviations for calcium as the dependent variable are designated in formulae by a subscript 2, thus, x_2', and for age as the independent variable by subscript 1, thus, x_1'. The product is entered with its sign in a small box at the corner of the individual cell. For example, the cell corresponding to 4.5 mg. per cent calcium and five to nine years contains a frequency (N) of 3 cases. Three times -2, which is (x_2'), equals -6; -6 times -2, which is x_1' equals $+12$. These products of each cell are also known as the product-moments and are summed for each horizontal row on the right side of the chart and separated in 2 vertical columns according to their plus and minus signs ($\Sigma + x_1'x_2'$ and $\Sigma - x_1'x_2'$). By subtracting the minus value from the plus value the final result of $\Sigma x_1'x_2' = +100$ is obtained.[8]

All values thus far obtained can now be entered into the "working formula," given in Table V, as used by Dr. E. L. Lucia (38), for the calculation of the correlation coefficient, r. In order to be successful in this rather complicated procedure, the investigator has to be thoroughly familiar with the meaning of the symbols. As we have determined the correlation of age and calcium, the value is designated r_{12} and is read "r one on two."

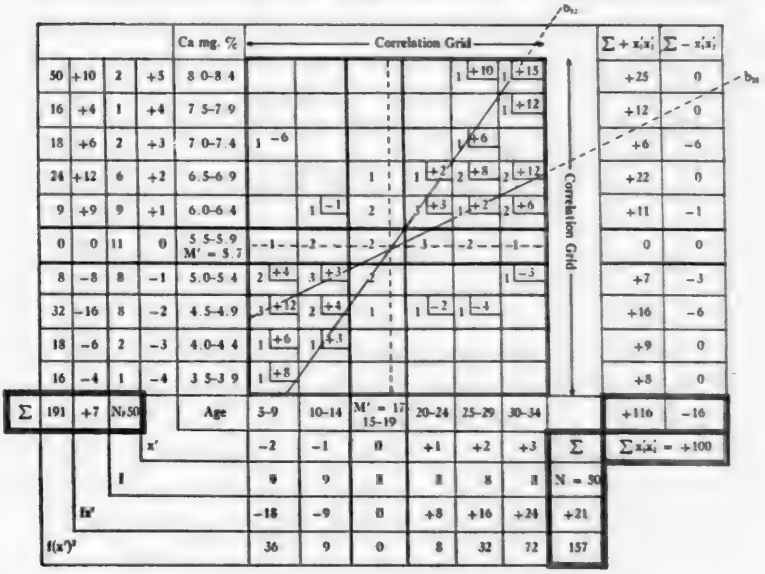
The final result for r_{12} equals $+0.579$ which indicates a positive (or direct) correlation between salivary calcium content and age.[9] The

[8] This calculation can be checked as to its accuracy by adding the products of the vertical columns. The final vertical total must equal that just obtained for the horizontal rows ($+100$).

[9] Again the fact has to be emphasized that these findings are based on arbitrary figures selected for demonstration only and they are not to be interpreted as being of biological importance.

TABLE V:

Correlation Grid Showing Computation of Correlation Coefficient for Ca mg.% and Age (from arbitrary origins)



correlation coefficient r_{12}

$$= \frac{\frac{\Sigma fx'_1x'_2}{N} - \left(\frac{\Sigma fx'_1}{N}\right)\left(\frac{fx'_2}{N}\right)}{\sqrt{\frac{\Sigma f(x'_1)^2}{N} - \left(\frac{\Sigma fx'_1}{N}\right)^2} \cdot \sqrt{\frac{\Sigma f(x'_2)^2}{N} - \left(\frac{\Sigma fx'_2}{N}\right)^2}}$$

$$= \frac{\frac{+100}{50} - \left(\frac{+7}{50}\right)\left(\frac{+21}{50}\right)}{\sqrt{\frac{191}{50} - \left(\frac{+7}{50}\right)^2} \cdot \sqrt{\frac{157}{50} - \left(\frac{+21}{50}\right)^2}}$$

$$= \frac{2 - (0.1400)(0.4200)}{\sqrt{3.8200 - (0.1400)^2} \cdot \sqrt{3.1400 - (0.4200)^2}}$$

$$= \frac{2 - 0.0588}{\sqrt{3.8200 - 0.0196} \cdot \sqrt{3.1400 - 0.1764}} = \frac{1.9412}{\sqrt{3.8004} \cdot \sqrt{2.9636}}$$

$$= \frac{1.9412}{1.95 \cdot 1.72} = \frac{1.9412}{3.3540} = +.579$$

statistical significance of the degree of correlation will be discussed under the standard error of r. This correlation can be observed *visually*[10] upon dividing the grid into quarters by running lines (dotted lines in the correlation grid) horizontally and vertically through the point corresponding to the M of all ages and the M of all calcium values, approximately in the center. It is seen that two of the quarters (at opposite corners) are practically vacant or especially devoid of extreme values and that the other two quarters (at the other two opposite corners) contain almost all of the cases. The cases fall in an arrangement such that the lower calcium values are in the lower age groups and vice versa; this indicates a strong relation between age and calcium. The greater the tendency for calcium and age values to fall along a straight, narrow line from one corner of the grid to the opposite, the higher the degree of correlation and the closer r will approach 1 (perfect correlation). In this example the correlation is *positive* (r approaches +1, "perfectly positive").

If however, the *low* age groups showed *high* calcium values the line of means would connect the previously vacant corners and the correlation would be very great but *negative* or inverse (r approaches −1); r never exceeds 1 and varies between +1 and −1. If the values of the grid lie not on a narrow line but are spread widely from the line (large standard deviations) r will decrease toward 0 or lack of correlation.

The correlation grid of Table V is used for two purposes, 1. for the calculation of various values for r; 2. for visual demonstration of whether correlation exists or not, by lines. This visual demonstration of a possible correlation is done by determination of so-called "regression lines" (Galton). One differentiates between "observed" and "calculated" regression lines the latter is also expressed as "line of best fit." The observed regression line is usually a zig zag line obtained by connecting the calculated means of each vertical column in the correlation grid, one might call these lines the "lines of means." A straight "line of best fit" is very often desired in graphic presentations. This is best determined by establishing the "calculated regression lines" as demonstrated in Table V because it is not always possible to estimate the position and slope of the observed regression line because personal

[10] A chart showing the individual cases plotted as dots on the grid (gunshot chart, (31)) can also be used for a visual survey.

error would be introduced and different workers would not obtain the same line for the same values.

To obtain the calculated regression lines it is necessary to calculate the "coefficient of regression" (b_{12}, read b one on two; and b_{21}, read b two on one) which follows the formulae below:

$$b_{12} = r_{12}\frac{\sigma_1}{\sigma^2} = (+0.579)\left(\frac{8.60}{0.98}\right)(+0.579)\ (8.7755) = +5.081 \text{ years.}$$

$$b_{21} = r_{12}\frac{\sigma_2}{\sigma_1} = (+0.579)\left(\frac{0.98}{8.60}\right) = +0.066 \text{ mg. per cent.}$$

The result of $b_{12} = +5.081$ years expresses the correlation between low age and low calcium, and high age and high calcium by saying that a unit increase in calcium (1 mg. per cent) is accompanied by an increase in age of 5.081 years on the average. Similarly, $b_{21} = +0.066$ mg. per cent indicates that a unit change of age (1 year) is accompanied by an increase of 0.066 mg. per cent, on the average for calcium.[11] After the regression coefficients have been calculated they can be used for another purpose namely to predict the value of one variable if the other one is known. If for instance the age of an individual is known to be between 20 and 24 years of age (Table V) one can approximately see by following the vertical column over the 20 to 24 age group that the calcium value must fall somewhere near the b_{12} line crossing through that column which is approximately between 5.5 and 6.4 mg. per cent.

If the observed regression line indicates that it deviates definitely from a straight line and follows more or less a curve of varying shape we are not dealing with a linear regression any more but rather with a curvilinear regression. The calculation of the curvilinear regression line depends on the appearance of the "observed regression line," which has to be established first. If it is then found that it follows a curve corresponding to a "second order parabola" or a "logarithmic" curve these may be calculated according to Pearl (49, pg. 332). There is no way of predicting which type of line will best fit a particular set of data other than by the knowledge gained from experience. Most lines for biological material, however, have been found to correspond

[11] Another method of calculating the regression lines is by the method of least squares described by Pearl (49, pg. 332).

to the three types mentioned: straight line, second order parabolic curve, and the logarithmic curve.

These values establish only the shape of the calculated curve and do not determine the *degree* of correlation as r expresses the correlation coefficient for a linear curve. It is sometimes extremely difficult to estimate whether there is any correlation or not and for this reason a biometrical figure expresses more adequately a possible relation

TABLE VI

Correlation Grid Showing Computation of Correlation Ratio for Non-Linear Tables

		Age										
	Ca MG. PER CENT	5–9	10–14	15–19	20–24	25–29	30–34	$N_2=f$	M_{1_2}	$M_{1_2}-M_1$	$(M_{1_2}-M_1)^2$	$N_2(M_{1_2}-M_1)^2$
	8.0–8.4					1	1	2	29.50	+10.40	108.1600	216.32
	7.5–7.9						1	1	32.00	+12.90	166.4100	166.41
	7.0–7.4	1				1		2	17.00	−2.10	4.4100	8.82
	6.5–6.9			1	1	2	2	6	26.17	+7.07	49.9849	299.91
Ca	6.0–6.4		1	2	3	1	2	9	22.56	+3.46	11.9716	107.74
	5.5–5.9	1	2	2	3	2	1	11	19.73	+0.63	0.3969	4.36
	5.0–5.4	2	3	2			1	8	14.50	−4.60	21.1600	169.28
	4.5–4.9	3	2	1	1	1		8	13.88	−5.22	27.2484	217.99
	4.0–4.4	1	1					2	9.50	−9.60	92.1600	184.32
	3.5–3.9	1						1	7.00	−12.10	146.4100	146.41
	$N_1=f$	9	9	8	8	8	8	50				1,521.57

Calculation of η:

$$\eta_{12} = \frac{\sqrt{\frac{\Sigma[N_2(M_{1_2}-M_1)^2]}{N}}}{\sigma_1} = \frac{\sqrt{\frac{1,521.57}{50}}}{8.60} = \frac{\sqrt{30.43}}{8.60} = \frac{5.52}{8.60}$$

$$= 0.642$$

likewise:

$$\eta_{21} = 0.583$$

between two variables of a curvilinear type and this value is expressed as the correlation ratio, η.

E. *Correlation Ratio.* The *correlation ratio*, η, (devised by Pearson) as stated above expresses the degree of correlation existing in the case of a curvilinear relation of two variables. Growth curves, for example, do not follow a straight line and the coefficient of correlation would not express the real extent to which growth and age might be related.

For this purpose η must be calculated. The rate of growth is very rapid at early ages but decreases as maturity is approached. The curve plotted for this relationship is very steep at first and then gradually levels off to no further growth, however, up to this point there is a high correlation, which is expressed completely only by η.

The formula and calculation of η are given in Table VI; the correlation grid is the same as in Table V as far as the dstribution and. number of cases is concerned. The following values have to be established for substitution in the general formula (right of heavy vertical line Table VI): 1. Mean age (M_{1_2} for each calcium group. 2. Difference between group means (M_{1_2}) and the total mean (M_1) of all age values: ($M_{1_2} - M_1$). 3. Square the difference: $(M_{1_2} - M_1)^2$. 4. Multiply the squared difference by the number of cases (N_2) in the individual age groups: $N_2(M_{1_2} - M_1)^2$. 5. Sum the results of step 4: $\Sigma N_2(M_{1_2} - M_1)^2$.

These values are substituted in the formula as follows: the square root of : the sum of all the values for frequency (N_2) times $(M_{1_2} - M_2)^2$ divided by the total number of cases (N) is divided by the standard deviation of the distribution of all cases according to age (σ_1). These steps are carried out under Table 6 and give the correlation ratio of age and calcium as $\eta_{12} = 0.642$. The values which η may take lie between 1 (perfect correlation) and 0, (lack of correlation) all being of the plus sign. In the above example the value of 0.642 indicates definite correlation between age and calcium which, in degree, is very close to that of the correlation coefficient, $r = +0.579$. The significance of any difference between η and r and whether the data fits better a linear or curvilinear regression will be discussed under the section on standard errors. The correlation ratio should be calculated again by reversing the position of the calcium and age values and their scales thus determining the η for calcium and age which is found for the cases of Table 6 to be $\eta_{21} = 0.583$. It is usually observed in linear correlations that r and the 2 η's are approximately the same, as in this example, however, in curvilinear correlations r and one η will be low (perhaps 0.01 or 0.10) and the other η will be great enough to show correlation.

F. *Standard Error*. For purposes of comparison the statistical measures, such as M, σ, and r, must be accompanied by some indica-

tion of their reliability. This gives, in other words, some idea of the probable scatter of the observed values (M, etc.) of different group investigations of approximately the same N about the "true value" of the entire universe ("true M". etc.).

It was stated earlier that this scatter could be reduced greatly by increasing N (e.g. from 50 to 500). The scatter of means, is however, not reduced directly as the size of the samples are increased, i.e. $\frac{50}{500}$ or to 1/10th, but has been shown to vary as the proportion of the square roots of the samples (24), thus $\frac{\sqrt{50}}{\sqrt{500}}$ or 1/3rd. Formulas have been devised on this basis for the calculation of the theoretical range of this scatter of group means about the "true mean." The resultant value is designated as the *standard error* or "standard deviation of the mean" (not to be confused with the standard deviation of the distribution of cases within the sample, see page 10). The symbols for the standard errors uniformly consist of a "σ" and as a subscript the symbol of the special term; for instance, the standard error of the mean and its formula are written $\sigma_M = \frac{\sigma}{\sqrt{N}}$.[12]

In practice the standard error of the mean is calculated by dividing the standard deviation of the sample by the square root of the number of cases. Just as the standard error of the mean expresses the probable fluctuation of the group means about the "true mean" so have standard errors of all the measures been devised to express their probable fluctuation about the "true measure."

The standard errors express the expected fluctuation of statistical measures only in terms of probability and cannot be used to set definite limits beyond which values could not occur due to the influence of chance in sampling. Most frequently not ± 1 but rather ± 2 standard errors are used to determine the accuracy of a given value.

[12] The standard errors of other statistical measures and their formulas (11) are as follows:

Standard error of the standard deviation $= \sigma_\sigma = \frac{\sigma}{\sqrt{2N}}$.

Standard error of the coefficient of variation: $\sigma_v = \frac{v}{\sqrt{2N}} \sqrt{1 + 2\left(\frac{v}{100}\right)^2}$.

Standard error of the coefficient of correlation: $\sigma_r = \frac{1 - r^2}{\sqrt{N}}$.

This range includes approximately 95.45 per cent of all values. It is the belief of many biometricians that greater allowance must be made for fluctuations due to chance in sampling, especially because of the skewness found in so many distributions. They suggest a range of ± 3 standard errors as giving greater allowance for chance errors. The final choice must be made by the individual investigator upon a thorough survey and analysis of his material (12). Some data require a greater allowance for error than others, for example, in establishing the dose of a toxic drug a worker would not want to take one chance in a million of giving a lethal dose while other material may be more satisfactorily tested by a range of $\pm 2\sigma$.

The most useful application of the standard error is found in the test for the "significance of a difference." Very frequently the mistake has been made in the literature in comparing established means for body fluids of healthy patients with those having pathologic lesions that great significance was attached to a slight deviation in results. Very seldom did investigators test whether the difference of results fell within the range of experimental error, or was the result of a small sample with a high value for the standard deviation. In practical terms they did not determine whether the results in pathologic cases were really significant for the disease as it has been established for instance that a high serum calcium level in most instances is definitely significant for hyperparathyroidism.

The value of the standard error can be used to determine the significance of a difference. In salivary work we might for instance want to compare the mean calcium values of two different age groups and see whether the difference between these means is significant for age. In this example twenty-six cases from 7 to 19 years and twenty-four cases from 20 to 24 years of age may be used. These are the same cases as found in Table V. The fundamental values to be established first are the mean (M), standard deviation (σ) and standard error of the mean (σ_M) for both age groups:

7–19 yrs.: N = 26; M = 5.31 mg% Ca; σ = 0.78 mg%;

$$\sigma_M = \frac{\sigma}{\sqrt{N}} = 0.153 \text{ mg\%}.$$

20 34 yrs.: N = 24; M = 6.31 mg% Ca; σ = 0.89 mg%;

$$\sigma_M = 0.182 \text{ mg\%}.$$

For the sole purpose of describing the accuracy of the mean, the

standard error usually accompanies it and is indicated by a plus and minus sign; as for instance M = 5.31 ± 0.153 mg. per cent.

In proceeding with the calculation to determine the significance of the difference the standard error of the difference between the two means of groups which are uncorrelated is to be calculated as follows:

$$(M_1 - M_2) = \sqrt{\sigma^2_{M_1} + \sigma^2_{M_2}} = \sqrt{(0.153)^2 + (0.182)^2} = 0.238 \text{ mg. per cent.}$$

If now by mere subtraction of the two arithmetic mean values, 6.31 − 5.31 = 1.00 mg. per cent, the result is at least three times the standard error of the difference (0.238 mg. per cent) it can be concluded that there is a definite significance of difference between these two means. In this example it will read:

$$\frac{M_1 - M_2}{\sigma_{(M_1 - M_2)}} = \frac{1.00}{0.238} = 4.20\sigma$$

This value of 4.20σ has been calculated on the basis of only fifty cases. Statistical experience has established that if a significant value for two means has been found to be as great as four times the standard error of their difference such a difference between means would occur by chance alone in approximately 0.6 times among ten thousand similar means (54) which is more rare an event than the approximately once in one hundred times ordinarily required for significance (three times the standard error).

The practical use of this formula is restricted if the two samples being compared are correlated. This would have been the case in the previous example if the individuals of the 7 to 19 age group had been examined for salivary calcium again several years later when they would all be in the 20 to 34 age group. Under these conditions there might be some correlation for the same individuals, between high Ca in the first age group and high Ca in the second and a correlation coefficient must be calculated. This is done by constructing a grid on which is plotted a point for each case. The point represents the first and second calcium values of the cases. The scale for the first value is placed on one axis of the grid and for the second on the other axis. The formula for the standard error of the difference, the significance of which is determined as above, becomes:

$$\sigma(M_1 - M_2) = \sqrt{\sigma^2_{M_1} + \sigma^2_{M_2} - 2r_{12}\frac{\sigma_1\sigma_2}{N}}$$

The standard error can also be applied to the establishment of the significance of differences of other values. It also is frequently used to find out whether a single correlation coefficient (r) is significant or not. In Table 5 it has been demonstrated that an approximate correlation between age and calcium content of saliva exists. It was stated at that time that the correlation coefficient was +0.579 for these fifty cases and that the closer r approaches one the more perfect the correlation. A definite borderline between significant and not significant coefficients of correlation cannot be estimated from the calculations of Table 5 but has to be established according to the following formula:

$$\sigma_r = \frac{1}{\sqrt{N-1}} = \frac{1}{\sqrt{50-1}} = 0.143.$$

$$\frac{r}{\sigma_r} = \frac{0.579}{0.143} = 4.05\sigma.$$

Here again if the result of the coefficient of correlation divided by its standard error exceeds three, then, r is definitely significant.

The standard error is also used often to establish by calculation whether the data of a correlation grid follow an "observed" regression line of a linear type or of a curvilinear type. For this purpose Blakeman's criterion of linearity can be employed. The calculation follows the formula below:

$$\zeta = \eta^2 - r^2 = 0.583^2 - 0.579^2 = 0.340 - 0.334 = \pm 0.006$$

The standard error of this criterion of linearity (ζ) is then calculated which follows the formula:

$$\sigma_\zeta = 2\sqrt{\frac{\eta^2 - r^2}{N}} = 2\sqrt{\frac{0.006}{50}} = \sqrt{0.0001} = 2(0.01) = 0.02$$

If ζ is greater than three times its standard error the regression line is curvilinear, and vice versa. In this instance ζ equals 0.006 and is very much smaller than its standard error 0.02 and therefore the regression line is of a straight or linear type.

In some publications the *probable error* (PE) has been used in the place of the standard deviation and the standard error. In the first instance it describes the measure of the dispersion of values about the mean in a frequency distribution. A range of M ± 1 PE contains

fifty per cent of all cases. It is calculated from the standard deviation by multiplying it by 0.6745. The PE offers no great advantage over σ. A range of $\pm 2\sigma$ corresponds to ± 3 PE. In the second instance the probable error has been used to express the reliability of the various statistical measures and, as with the standard error, the designation would then be the probable error of the mean, of the coefficient of correlation, etc. These are also calculated merely by multiplying the particular standard error by 0.6745. The probable error is therefore not as useful as the standard error and offers no advantage over it in the field of salivary analysis.

G. *Differences between Proportions and the Chi Square* (χ^2) *Test.* Many dental problems arise wherein success or failure are the only results possible, for instance, patients might be treated because of a high incidence of dental caries. As a result the caries process may either be "arrested" (success) or continue (failure). The biometrical evaluation of experiments involving the effectiveness of treatments (or other "attributes" that express only two possibilities: presence or absence) require different formulas from those just described for measurements of composition, height and weight (or other "variables").

The percent of success attained by treatment must be tested by accepted formulas for the significance of the difference between the proportions of success to failure among treated and untreated cases. Significance is shown if the difference is greater than would be expected to occur by chance between different groups of patients of similar size selected at random from an untreated population (universe). If this experiment were carried out the following hypothetical results might be found:

	Treated	*Untreated*
Caries Arrested	25	10
Caries Not Arrested	30	40

The question arises as to whether an investigator would be justified in concluding on the basis of these results that the treatment was effective although not perfect or that such a proportion of success could have been obtained merely by the chance inclusion of many cases in the treated group who would have shown "arrest" had they been allowed to remain untreated. One of the simplest ways of test-

ing this is by means of the ratio of the difference between the two proportions divided by the standard error of the difference, according to the following formulae in which p and p′ are the two proportions of success obtained by calculating the percentage of "arrested" cases in the totals (55 and 70) of the respective groups, q and q′ are the respective percentages of failure or continued caries:

$$\sigma_{(p - p')} = \sqrt{\frac{p \times q}{N} + \frac{p' \times q'}{N'}}$$

$$= \sqrt{\frac{45.2 \times 54.5}{55} + \frac{20 \times 82}{50}}$$

$$= \sqrt{45 + 32} = \sqrt{77} = 8.8$$

$$\sigma \frac{p - p'}{(p - p')} \frac{25.5}{8.8} = 2.9$$

The difference between the proportions is 2.9 times its standard error and the investigator would be able to state that the treatment had been effective in a greater proportion of cases than would probably occur by chance. The limit of significance is 3 standard errors as discussed under the previous section. The significance of this difference between proportions can be tested by several other formulae (24) but the one given above is applicable to a wide range of problems.

Another problem which frequently arises is the significance of a *series* of proportions. For example, in the above illustration there might have been a number of different treatments tested. The results, hypothetically, would show that all were partially successful but some more so than others and the question would arise as to whether the best one was significantly more successful than the least successful or whether the difference could have occurred purely by the chance inclusion of more or less cases which would have recovered without treatment. In order to test this series of differences between the proportions of success to failure the χ^2 value is determined. This test was originally devised by Pearson (24).

In this example many types of treatment could have been included but in order to simplify the illustration only the above results for treated and untreated groups will be given. The procedure and

calculations are the same as though several treatments had been included; an example with multiple treatments is given by Hill (24).

The following values (the paragraph numbers refer to both the upper and lower divisions of the table) are to be calculated and listed for the χ^2 test: 1. The observed numbers of cases in which caries *was arrested* in treated (25) and untreated (10) groups are recorded and a total (35) entered at the right. The observed numbers of cases in which caries was *not arrested* in the two groups (30 and 40) are recorded, and a total (70) is again entered at the right. Below, totals are entered for the

TABLE VII

χ^2 Test for Significance of Treatment "A" for Arrest of Dental Caries

	TREATED	UNTREATED	TOTAL
(1) Observed number in which caries *was arrested*	25	10	35
(2) Expected number on hypothesis that treatment and arrest of caries are unrelated	*18.3*	*16.7*	*35*
(3) Difference, observed minus expected	+6.7	−6.7	—
(4) (Difference)^2	45	45	—
(5) (Difference)^2 ÷ expected number	2.46	2.69	—
(1) Observed number in which caries was *not arrested*	30	40	70
(2) Expected number on hypothesis that treatment and arrest of caries are unrelated	*36.7*	*33.3*	*70*
(3) Difference, observed minus expected	+6.7	−6.7	—
(4) (Difference)^2	45	45	—
(5) (Difference)^2 ÷ expected number	1.23	1.35	—
Total — Observed	55	50	105
Total — Expected	*55*	*50*	*105*

number of cases treated (55) and untreated (50) and a grand total (105) at the lower right. 2. The *expected* numbers (*18.3*, *16.7*, *36.7*, and *33.3*) of cases in each of the four categories are calculated, on the hypothesis that treatment and arrest of caries are unrelated, by multiplying the observed number by the percentage of "arrested" cases in the totals column: $\frac{35 \times 100}{105} = 33.3\%$; 33.3% times $25 = 18.3$. The expected values are italicized in Table VII. 3. The differences between observed minus expected values for all four groups are entered

next. 4. This difference is squared. 5. The squared difference is divided by the expected number.

Finally the values of line (5) of the table are totaled (2.46 + 2.69 + 1.23 + 1.35) and are found to equal 7.73, this sum is known as the χ^2 value. To make use of χ^2 a new "n" must be calculated by the formula below in which R is the number of horizontal rows which give "observed" values not including the row of totals, and C is the number of vertical columns containing "observed" values not including the column of totals.

$$n = (R - 1)(C - 1) = (2 - 1)(2 - 1) = (1)(1) = 1.$$

The values of χ^2 and n are now located in the tables of χ^2 of Fisher (16, 24, 61) and opposite them for the present example we find the probable occurrence of such a difference between proportions of success would occur less than one time in one hundred random samples. This result confirms the significance noted by the previous test based on the standard error of the difference. The χ^2 test is widely applied in biology and medicine and its possibilities can be readily observed.

In conclusion it can be stated that the application of these described biometrical procedures offer the possibility of new methods of investigation and new interpretations of established data in dental science. Many correlations have appeared in the dental literature which have not been tested for sampling errors as is required for their acceptance in all fields of science. Many times the theories of dental caries have been investigated on a large scale, but there are only one or two instances where biometrical methods have been applied. The difficulties of technical procedure in biometry are often very trying, especially in the tabulation and analysis of the data.

AIDS IN BIOMETRICAL ANALYSIS

For a comparatively small number of cases it is possible to evaluate all master and individual records by transcribing the original data onto special tables (in ascending order, etc.). This procedure even if handled by very dependable persons does not exclude the possibility of individual errors during transcription as well as in calculation. If a large amount of material, for instance over five hundred cases, are to be recorded and evaluated this procedure becomes so cumbersome and

time consuming (especially if all values are to be checked twice or three times) that it is necessary to resort to mechanical aids which will increase both speed and accuracy.

It hardly has to be emphasized that the slide rule is indispensable in statistical work and in the biometrical evaluation of several thousand salivary analyses made in this laboratory it even was noticed that the slide rule had to be supplemented by an electric calculating machine, mathematical tables (2, 46, 48), and a card system which proved to be of utmost value for greater speed and accuracy of the

CASE RECORD CARDS FOR HAND SORTING

		Name T------, T.	♀ Age 8
Case #		1	
Date		4-34	
A	Ca	mg%	5.0
B	P	mg%	15.0
C	cc/hr		21.0
D	Ca	mg/hr	1.07
E	P	mg/hr	3.21
F	CaXP	mg%	75.0
G	Ca/P	mg%	0.33
H	CaXP	mg/hr	3.43
I			

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FIG. 3

above calculations. Because of the volume of computations every advantage has to be taken of these efficient aids. The card system in particular allows a very quick sorting of the material. The data are transferred onto cards (3 x 5") each being identified by its case number as illustrated in *fig. 3*. It is preferable to have these cards printed according to a definite form so that each particular item is in an invariable position. The name, in the illustration, can always be found at the center of the first line and by arranging all cards in alphabetical order they can be checked for errors or duplication of cases. The sex and age are placed in an important position at the upper right as they

will be used in almost every evaluation. The case number at the left hand column is used only for identification and checking; the date may be required for some types of evaluation. The items following in the left hand column are lettered according to the alphabet providing ready identification of the various analyses and notations of the case. A code identification is to be preferred because the entire card, front and back, can be printed and ruled at once and newly observed items added to the card in any desired order; also an assistant unfamiliar with biochemical or dental terms is less likely to read values incorrectly. Letters rather than numbers are used because no confusion can then arise between the symbol and the numerical observations themselves.

Cards of the type illustrated may be used over and over again simply by rearranging their order. After checking for duplication in alphabetical order, the distribution according to age groups, sex, season, salivary composition, caries frequency, etc. etc., can be made in any combination, all with the same set of cards. On the other hand, if cards are not used observations must be transferred from the master record onto tables which list the case numbers according to the order of the particular item. This procedure is not only time-consuming and difficult to check, but must be *repeated completely* for each new observation studied.

When arranging the printing of these cards, it will be of advantage to consider the use of colored cards. For example, the cases may be roughly divided into 20-year age groups (or some other important classification), using cards of a distinctive color for each group. A large number of cards can be more easily sorted and misplaced ones quickly discovered.

After the entries have been typed on the cards, they must be checked *very carefully* against the master record. One person should be in charge of this, and should make a complete check himself at least once, and then supervise a second check by others. This same person should be in charge of all sorts made and should check the card count before and after each rearrangement.

The outstanding application of this card system is in preparing the data for entry in a correlation grid. They provide the only dependable method of filling in a correlation grid if more than 50 or 100 cases

are involved. An example of the type of record made by hand-sorting fifty cases for entry in a correlation grid (Table V) according to their age and calcium content is given in *fig. 4*.

This tally count may be prepared by marking the appropriate cell on the scatter grid as each card is passed; however, a more accurate method is to rule off the cells of the grid on a piece of wrapping paper large enough to lay out the cards themselves. After setting out all of the cards, the stacks are counted and the total of each cell entered

TALLY COUNT ON SCATTER GRID

AGE

Ca mg.%	5	10	15	20	25	30	f
8.0					I	I	2
7.5						I	1
7.0	I				I		2
6.5			I	I	II	II	6
6.0		I	II	III	I	II	9
5.5	I	II	II	III	II	I	11
5.0	II	III	II			I	8
4.5	III	II	I	I	I		8
4.0	I	I					2
3.5	I						1
f	9	9	8	8	8	8	50

FIG. 4

in a smaller diagram made on graph paper. The cards are not taken up until checking is completed. It is in this latter operation that the card system is indispensable because errors in sorting are promptly located by checking the totals of rows and columns.

Another use for these very important cards is for the preparation of a second set which are required if machine sorting is to be employed. Hand-sorting becomes less feasible as the number of cases increase. If several hundred cases are to be studied the time consumed in sorting and checking becomes prohibitive. It is possible to employ

machine sorting by means of the Hollerith cards which are available in most large cities.[13] As these are punched according to the data to be entered, a somewhat different form may be found more useful for the 3″ x 5″ index card from which the Hollerith cards are punched. A suggested form is given in *fig. 5* (the data is the same as that of *fig. 3*). The new card must contain zeros filled out to the total number of digits of the highest value of each variable occurring in the total number of individuals. For example, the case number is now written as 0001 because the total number of cases is more than 999 and less than

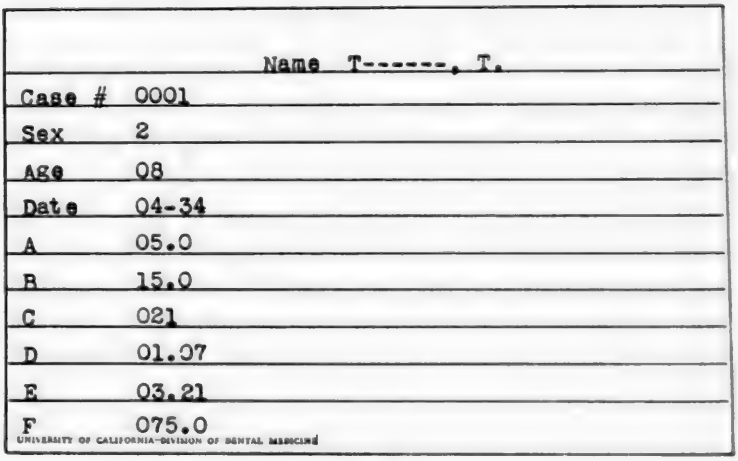

FIG. 5

9,999 and the Hollerith card, which is illustrated in *fig. 6* has to have all blanks punched as 0.

The standard form illustrated in *fig. 6* consists of a $3\frac{1}{4}$″ x 7 3/8″ card on which are printed 80 columns of figures each from 0 to 9. These 80 columns are to be divided into fields according to the number of digits in the values to be punched. In the illustration, the field set aside for the group of columns for case number is punched 0001. The

[13] International Business Machines Corp., 25 Battery St., San Francisco.

cards can be arranged and counted in the order desired by means of the sorting machine (at the rate of 400 per minute). The form illustrated was especially printed on a blank card, obtained from the punch card company, which originally contained only the eighty columns of numbers. This can be printed at no additional expense at the factory. For convenience in checking, a printed form is of great assistance, as each field can be readily found and the fine vertical lines indicate the position of the decimal point. Visual checking is greatly facilitated by requesting that the figures for each individual value punched be automatically printed along the top of the card by

PUNCH CARDS FOR MACHINE SORTING

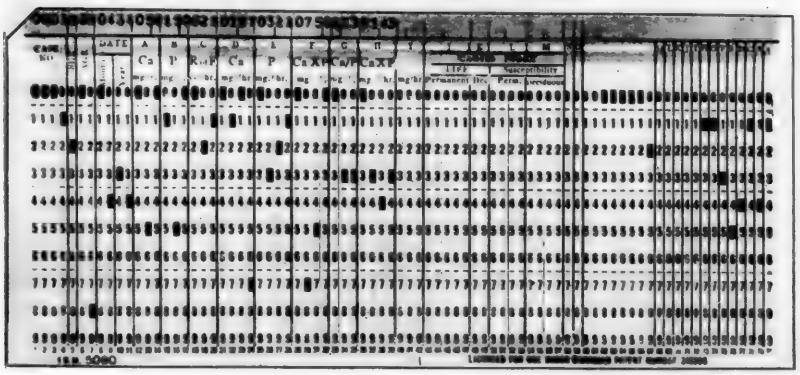

FIG. 6

means of the printing punch. Neither the printed form nor the printing punch are essential to the success of the method because each individual field can be identified by the column numbers at the bottom of the card. In arranging the printed form, a symbol (letter or number) for each field is of aid in arranging instruction for the sorts and tabulations to be made by the punch card company. A typical instruction form might be given as: "Sort according to 5-year age-groups and $\frac{1}{2}$ mg. intervals (as 3.0–3.4, 3.5–3.9 etc.) with the major control on age and minor control on field A." A consultation with a representative of the punch card company should be arranged before the forms are made, as suggestions for handling special conditions can

be given. Sorting and tabulating (see below) are greatly facilitated wherever step intervals are chosen which are not units, tens, hundreds, etc. (e.g. 5 or 3), by punching the card in a special column according to its order among the step interval values. This procedure which is called gang punching has been carried out in columns 62 to 80, of the card illustrated in *fig. 6*.

In addition to the ten punching positions indicated in each column, two extra ones are available. These are called "11 and 12" and are situated immediately above the zero in each column. The 12 position is above, followed by 11 (also designated "x"), and then the 0, 1, 2, etc. These extra numbers are of great value where dates, according to the month, etc., are to be punched and extra columns are not available.

Printed reports can also be obtained from the punch-card company, these are made by the tabulating machines at the rate of 150 cards per minute. The beginning of a more complicated report is shown in *fig. 7*. These are prepared after the sorting machines have arranged all cards in order according to the correlation desired. A tabulating machine will record individual values according to sex, age group, and salivary composition, with individual totals, sub-totals, group totals and grand totals of the number of cases.

It will be noted in *fig. 7* that the tabulating machine does not record the decimal point or the titles of the columns of figures. These designations must be entered by the investigator at the top of the columns. The first column of *fig. 7*, containing "2" 's only, indicates the sex (female) the second gives the age by five year groups. The subdivisions of the age group 5 to 9 years extend on down to the last three rows where the values for the 10 to 14 year group start. The third and fourth columns beginning with 030 and 004 represent values for two components of saliva. The fifth indicates the number of cases in each individual group divided according to the step intervals (003 to 005, 006 to 008, 009 to 011, etc.) of the values in column 4. The sixth gives a subtotal of the number of cases of all values of the second component (column 4) which also have a value of the first component within the range of the step intervals (030 to 034, 035 to 039, etc.). The seventh column indicates the group total of cases from 5 to 9 years. Another column to the right, not shown in *fig. 7*, will give, at

the end of the report, the grand total or number of cases of the female sex. The numbers printed under columns 2, 3, and 4 are not necessarily the lowest value of the cards in the particular group or step interval. The value printed is that contained on the top card of the group and hence is always a number falling within the group or inter-

```
2    6 030 004          1
                                    1
2    7 035 004          1
2    7 039 008          1
2    6 035 021          2
                                    4
2    8 041 003          1
2    9 042 010          2
2    7 044 018          2
2    6 044 022          1
                                    6
2    6 049 005          4
2    7 046 010          3
2    8 047 034          2
                                    9
2    9 052 007          2
2    7 054 013          1
2    7 052 015          2
2    5 050 020          2
2    9 053 030          1
                                    8
2    6 055 012          1
2    9 059 018          1
2    9 056 024          1
                                    3
2    8 062 006          2
                                    2
                                              33
2   10 034 011          1
                                    1
2   10 039 014          1
2   14 036 017          1
                                    2
```

FIG. 7 Tabulating Machine Report

val being counted. For example, if there were more than one case in the first group the value under column 4 could still be 004 instead of 003 if that particular card were on top.

The accuracy of reports prepared by this method depends solely upon the human elements involved. If the original records from which

the Hollerith cards are punched are correct, and the latter are verified (a mechanical method of checking after punching), no mistake is possible by the machines. The cost of machine sorting is moderate considering the saving of labor, the accuracy attained, and the great speed with which the reports are completed.[14]

In closing it must be stated that there are of course still other phases of biometry which have not been covered. The purpose of this paper is to introduce some of the applications to dentistry. The texts of Hill (24), Pearl (49), Woods and Russell (60), Dunlap and Kurtz (11), and Yule (61) supply the fundamentals of medical biometry and especially point out common fallacies and mistakes which can be made. The publications of Fisher (15, 16), Jeffreys (27), and Wilson (58, 59) emphasize other precautions to be considered in the general applications of "statistical inference."

SUMMARY AND CONCLUSION

The many etiological problems still unsolved in the clinical fields of dentistry and medicine and the tabulations of clinical data offered in the literature to show the association of oral lesions and systemic disorders have brought forward more forcibly than ever the necessity for biometrical evaluation of clinical data. Many investigators in dental science have spent years in accumulating clinical data without taking advantage in their final reports of methods which will place the results before the profession in a more scientific light and which would increase the practical value of individual results. Many false and far reaching conclusions could have been avoided if they had followed the principles of biometry which admittedly require special study and consume much time.

It is often felt that biometrical procedures are unnecessary and that biological laws and conditions cannot be solved by their use. This concept is found frequently among overenthusiastic clinicians and

[14] The total charges in a representative analysis of 650 cases, requiring twelve complicated reports, amounted to approximately $70.00 (including purchase of cards, printing, punching, verifying, sorting, and tabulated reports), of which only $30.00 was expended for tabulated reports. The cards, which are completely punched, will be available for future reports without further expense for printing, punching, or verifying. These reports were made in the "Service Bureau" of the punch card company. Suggestions for applications of the punch-card method are to be found in the following references: Baehne (1), Martin (43), and Pearl (49).

biologists. It must be regretted that biological science has made so little use of this most efficient means of expressing clinical and laboratory observations in terms which can be checked and used in many ways. Biometrical procedures are by no means new and dentists and physicians should make just as much use of them as do anthropologists, governmental administrators, educators, public health officials, and business executives.

How often has it happened in dentistry and medicine that correlations of factors have been published without applying these biometrical methods? Some of the most typical examples in dentistry may be quoted: the high frequency of paradentosis in diabetes which is found in all text books of dentistry and which has been taught for many years; an antagonism between acidity and alkalinity of urine and saliva (21, 22); the slogan that 4 out of 5, that is 80 per cent of the total population, are suffering from paradentosis; that the presence or absence of bacillus acidophilus is related to the frequency of dental caries (26); that the change of dietary regime will reduce caries frequency (8, 20, 25, 29); that the extraction of teeth with periapical infection is the only solution of our focal infection problem because of the high frequency of subsequent systemic disturbances (19, 23, 51). If all the data in these problems had been accumulated on a proper basis and evaluated biometrically, the authors venture that the conclusions might have appeared in an entirely different light.

The same holds true for the many publications which have appeared in the field of salivary composition. In the middle of the 20th century we are still unfamiliar with the importance which saliva might have in relation to teeth. To clarify some of the most fundamental problems of salivary composition, it was found necessary to delve into the field of biometry and to use these principles in the establishment of fundamental knowledge. It soon was found that only a limited number of procedures could be used and that these were the same as those which are applied in most routine biometrical evaluations in biological science. However, the time consumed in the study of all procedures and in determining which ones were most suitable, emphasized the need for a concise presentation of the most frequently employed methods.

The biometrical terminology alone presents many difficulties in

understanding these methods and for this reason the terms and their practical applications have been described in detail. This text by no means replaces the publications of Fisher (16), Hill (24), Pearl (49), and Yule (61), and is not intended as a "short cut" but rather as a simplified and condensed summary which will enable any clinician or laboratory worker in the dental and medical field to make use of his data in a much more efficient manner than has been done in the past.

The greater accuracy and completeness of description of results obtained by these procedures is an incentive which balances the difficulties of learning these methods. For these reasons most emphasis has been placed on the description of the practical methods. It has been pointed out that a large volume of experimental material is required if it is to be used for biometrical study. The methods of recording and the various aids in biometrical analysis, with regard to hand sorting and machine sorting, the calculation of the arithmetic mean, standard deviation, frequency distribution, histogram, and ogive, as the most fundamental tools are considered in detail, followed by the more complicated determinations of correlation coefficient, regression coefficient, correlation ratio, standard error, probable error, significance of differences, and the chi square test. The latter group, even though less frequently used, give really the interpretation of the former ones and provide a very descriptive presentation of the relations of the data. For each calculation, a typical example is given in the field of salivary analysis for which other problems can easily be substituted by the individual investigator. Wherever additional methods are employed in the establishment of salivary standards of composition a description of the technical procedure will be given.

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A STUDY BY AGE AND SEX OF THE INCIDENCE OF DENTAL CARIES IN OVER 12,000 PERSONS[1]

FRANKLIN HOLLANDER, PH.D.

School of Dental and Oral Surgery, Columbia University

AND

JAMES M. DUNNING, D.D.S.

Metropolitan Life Insurance Company, Dental Division

It is generally believed that dental caries is "essentially a disease of childhood," and that its incidence among adults is very low as compared with its pre- and post-pubertal onslaught. So widely is this view accepted that one can find a statement of it in the writings of most of the major investigators of dental caries [Pickerill (1), Bunting (2), Bödecker (3)]. In consequence, most of the systematic attempts to control decay in humans have been carried out with children. It is generally admitted, however, that this statement is based on *clinical impressions* rather than on any systematic investigation of a large representative group of people. A search of the literature on the incidence of decay among humans has revealed a considerable number of studies on children, but very few which cover the supposedly critical period of young adulthood. Furthermore, those studies which did extend beyond the age of 20 were, in all cases, carried out on dental patients—not on a representative cross-section of the entire population. For instance, Driak (4) has reported a study of 1100 Viennese patients in the age range 10 to 90 years, based on the number of obviously carious teeth present in each mouth. A "full diagnostic survey" on 1000 patients (ages 20 to 60) was reported by Cramer (5) and an even more extensive analysis of 2723 cases (ages 10 to 80) was reported by Brekhus (6) on the basis of extracted teeth only. Based wholly on the dental condition of individuals who came to the dentist for treatment, all three of these

[1] Presented at the Sixteenth General Meeting of the International Association for Dental Research, Minneapolis, March 12–13, 1938.

studies automatically exclude people with little or no decay. Thus, it must be concluded that the groups of people used in these several studies were not at all representative of the general population, so far as degree of dental caries is concerned; consequently, deductions drawn from them are no more applicable to the community as a whole than are deductions inferred from gross clinical impression.

STATISTICAL STUDY OF A TYPICAL GROUP OF 1,000 CLINIC PATIENTS

In order to demonstrate how erratic the usual studies of clinical material can be, we have undertaken an analysis of our own, similar to the above. For this purpose, we used data on 1000 patients of the white collar class, collected by the dental clinic of the Temporary Emergency Relief Administration in New York City, and published in *Health Dentistry for the Community* (7). The original records in this study give the number of unfilled cavities and the number of missing teeth in each mouth for each of the eleven five-year age groups in the total sample. In order to calculate caries incidence from these values we used the total number of cavities in each mouth, and followed Bödecker's procedure (8), by assuming that each missing tooth corresponds to a mean value of three cavities. The validity of this value is discussed below. Thus, to find the total number of cavities corresponding to each age group, we added the number of actual cavities to three times the number of missing teeth, and then divided the sum by the number of patients in that particular group. The resulting value of the mean number of cavities per person was plotted against the median age for each group and is represented by the solid line in the graph (*fig. 1*). The number of individuals in each of the age groups, 10–14, 60–64, 65–69, and 70 or over, was very small compared with the others; hence we have felt justified in omitting these groups from the present analysis, although their inclusion would in no way have affected the final result.

As can be seen from the graph, the data conform extremely well to a straight line, the slope of which corresponds approximately to an annual increase of 0.9 cavities per person per year of age. Since we are interested here only in showing to what common errors a study of this kind is liable, we have made no attempt to determine the best equation of the straight line. It suffices for our purpose simply

to point out its implications, namely, that the average rate of increase of dental decay (as measured by its slope) *appears to be independent of age.* The steady rise of the curve throughout the entire age range

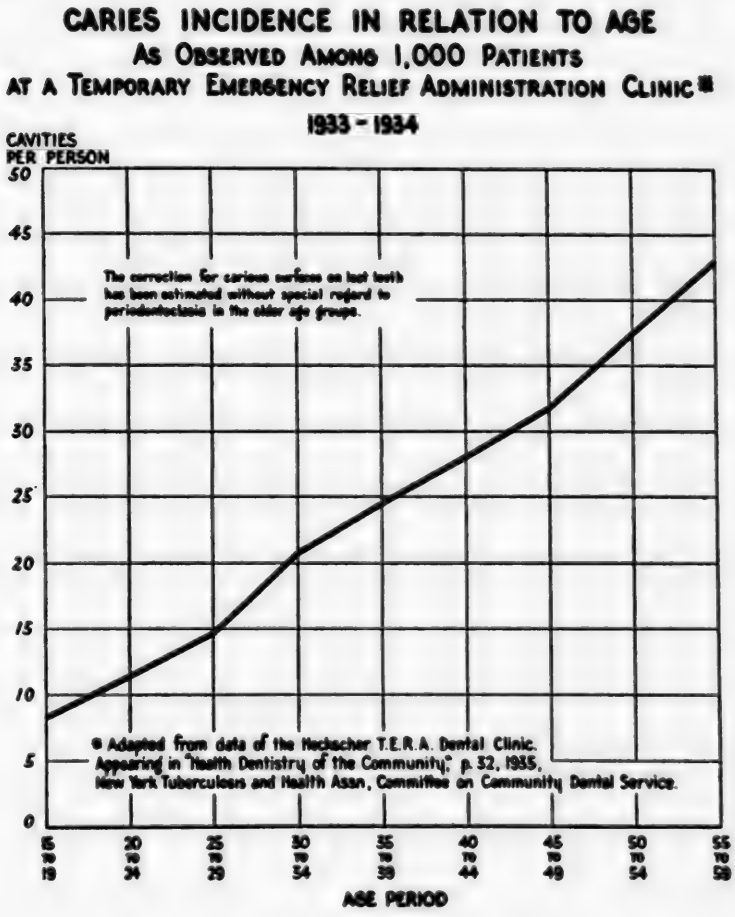
CARIES INCIDENCE IN RELATION TO AGE
AS OBSERVED AMONG 1,000 PATIENTS
AT A TEMPORARY EMERGENCY RELIEF ADMINISTRATION CLINIC*
1933 - 1934
CAVITIES PER PERSON
50
45
40
35
30
25
20
15
10
5
0
The correction for carious surfaces on last teeth has been estimated without special regard to periodontoclasia in the older age groups.
* Adapted from data of the Hochscher T.E.R.A. Dental Clinic. Appearing in "Health Dentistry of the Community," p. 32, 1935, New York Tuberculosis and Health Assn. Committee on Community Dental Service.
15 to 19
20 to 24
25 to 29
30 to 34
35 to 39
40 to 44
45 to 49
50 to 54
55 to 59
AGE PERIOD

FIG. 1

is a manifestation only of the cumulative nature of the disease. From this study, therefore, it would seem that the progress of dental decay manifests no pronounced critical period between 15 and 60 years of

age, thus contradicting the general clinical impression as well as the conclusions drawn from the statistical studies of Driak and Cramer.

The validity of the conclusion drawn from this straight line, however, is open to serious question for the two following reasons: In the first place, a careful examination of the source material reveals that the "number of cavities" included actual cavities only. No record is given of the number of fillings present at the time of examination. Consequently, what we have called "number of cavities per person" is only a crude measure of the total caries incidence. Although this includes part of the decay which had developed previous to the last dental treatment (i.e., that part included in "missing teeth"), and those teeth lost because of periodontoclasia, it omits that very considerable portion of the total accumulated decay corresponding to the fillings. Such an omission is a very serious one, for if it had not occurred, the graph might have shown a rising curve instead of a straight one. Secondly, it is only reasonable to expect that the rate of carious attack (i.e., rate of development of new lesions) must decline as the total number of attackable, but not yet affected, positions in the teeth of any one mouth diminishes. This follows purely as a matter of probability, since the number of such remaining attackable positions undergoes a steady diminution as new lesions develop with advancing age. Any curve, therefore, which fails to level off at all with advancing age is open to a certain degree of suspicion.

The outstanding characteristic of this group of people which differentiates them from the entire population is that they were patients reporting on their own initiative at a dental clinic, and patients, also, of a particular social stratum. It is to be assumed that all of them had caries of a degree sufficiently advanced so that they were well aware of the condition, and this fact makes it probable that the caries incidence found among them is different from that of the population at large.

The foregoing suggests that the results of any statistical study of caries incidence in dental patients is liable to vary with the particular group of patients chosen and the method used for measuring the degree of decay. In other words, the difference between our analysis of the TERA data and the studies of Driak and Cramer indicate that the dental condition of a group of *patients* is not typical for the

entire population of a community and therefore clinical material cannot be employed as a general measure of caries incidence. Consequently, in order to establish the existence of a critical age period of general physiological or developmental significance, the study must involve a group of individuals which is so large and so diverse as to constitute a reasonably good cross-section of the entire community.

A STUDY OF A HETEROGENEOUS GROUP OF 12,753 INDIVIDUALS

A set of data which possesses many of the above requirements has been made available to us.[2] For a number of years, it has been the practice of the Metropolitan Life Insurance Company to perform a thorough dental examination every six months on each of its employees, irrespective of position, salary or dental requirements. Using the complete records of one of these series of examinations in 1927, Hyatt employed this material for a study of caries incidence with reference to individual surfaces as well as individual teeth. Although he was not concerned with total caries incidence per person, his observations were recorded in such a form that this value could be readily calculated; also, age and sex data were included in the records. Since this material is so well suited to our purpose we have taken advantage of its availability to carry out the present analysis.

These dental examinations, covering a group of 12,753 individuals in the age range of 17 to 65 years, possessed the great merit, for our purpose, of having been made in a completely routine manner, regardless of the individual's desire or need for dental treatment. Furthermore, the group offers a reasonably satisfactory cross-section of society as found in New York City at this time, since it includes plumbers, bricklayers, carpenters, electricians, clerks, commissary workers, section heads, stenographers, executives, and professional workers. It also includes a variety of different races, but the proportion of married to unmarried women is very low as compared with the community as a whole. Most of the subjects are in the lower age groups, but even in the higher groups the samples are of sufficient size to warrant statistical consideration. The dental records indicate fillings, missing teeth, and cavities of all sizes, classified according to

[2] Through the cooperation of Dr. Thaddeus P. Hyatt and Dr. Alfred J. Lotka.

tooth surfaces involved. Details of dental and statistical procedure can be found in Hyatt's original report (9).

In order to evaluate the total incidence of dental caries in any one mouth, three different criteria were available to us: (1) the *total number of teeth* affected by caries, irrespective of the degree of decay [the DMF teeth of Klein and Palmer (14)]; (2) the *total number of tooth surfaces* affected, making an adequate allowance for the average number of such surfaces which correspond to a crown or a missing tooth; and (3) the *total number of lesions*, making similar allowance for crowned and missing teeth. Because of the form in which the dental records have been transferred to the punch cards, we found it impossible to estimate the number of actual lesions with any degree of satisfaction. The other two criteria, however—carious teeth per person and carious surfaces per person—could be estimated from these cards with a reasonable degree of reliability. A comparison by entire affected teeth seemed to possess the advantage of simplicity and accuracy, whereas a comparison by affected surfaces seemed to afford a more complete quantitative picture. Inasmuch as a comparison of the results obtained by two different criteria might prove to be of some interest, we carried out our analysis of these data in terms of both criteria.

The total number of decayed teeth in each age and sex group was determined by the formula:

$$\text{Total number of affected teeth} = (32 \times \text{no. of persons}) - (\text{no. of perfect teeth} + \text{no. of unerupted teeth}).$$

The number of decayed surfaces was determined by multiplying the number of teeth corresponding to each code number of Hyatt's tabulation by a conversion factor which represents the number of carious surfaces equivalent to the dental condition represented by that code number. The resulting products were added together to give the total number of affected surfaces in each group. These values are represented in Table I, as are also the corresponding values for affected teeth and the total number of persons in each group. Then, dividing the total number of carious teeth (or surfaces) by the total number of persons, we obtained the mean number of such carious teeth (or surfaces) per person in each age and sex group. The conversion

TABLE I

Caries incidence as measured by affected teeth per person and by affected surfaces per person, observed among home office employees of the Metropolitan Life Insurance Company classified by sex and age, 1927

AGE AND SEX	NUMBER OF PERSONS	AFFECTED TEETH		AFFECTED SURFACES	
		Number	Per person	Number	Per person
All Ages: Total	12,753	206,050		439,895	
Males	3,388	59,646		138,327	
Females	9,365	146,404		301,568	
17–19: Total	4,343	54,981	12.66	101,975	23.48
Males	566	6,763	11.95	12,640	22.33
Females	3,777	48,218	12.77	89,335	23.65
20–24: Total	4,427	70,017	15.82	139,517	31.52
Males	875	13,756	15.72	28,221	32.25
Females	3,552	56,261	15.84	111,296	31.33
25–29: Total	1,301	23,785	18.28	52,584	40.42
Males	446	7,892	17.70	17,836	39.99
Females	855	15,893	18.59	34,748	40.64
30–34: Total	757	15,305	20.22	36,372	48.05
Males	358	7,189	20.08	17,305	48.34
Females	399	8,116	20.34	19,067	47.79
35–39: Total	591	12,107	20.49	30,128	50.98
Males	318	6,211	19.53	15,362	48.31
Females	273	5,896	21.60	14,766	54.09
40–44: Total	432	9,335	21.61	24,150	55.90
Males	219	4,520	20.64	11,500	52.51
Females	213	4,815	22.61	12,650	59.39
45–49: Total	385	8,521	22.13	22,603	58.71
Males	248	5,271	21.25	13,815	55.71
Females	137	3,250	23.72	8,788	64.15
50–54: Total	250	5,590	22.36	14,995	59.98
Males	165	3,495	21.18	9,276	56.22
Females	85	2,095	24.65	5,719	67.28
55–59: Total	159	3,820	23.03	10,476	65.89
Males	107	2,488	23.25	6,735	62.94
Females	52	1,332	25.62	3,741	71.94
60–64: Total	65	1,519	23.37	4,081	62.78
Males	49	1,156	23.59	3,101	63.29
Females	16	363	22.69	980	61.25
65 and over: Total	43	1,070	24.88	3,014	70.09
Males	37	905	24.46	2,356	68.54
Females	6	165	27.50	478	79.67

factors and their significance are also given in Hyatt's report. Only one of them requires specific mention here: the factor chosen for both missing and crowned teeth, which is 3 surfaces per tooth. This

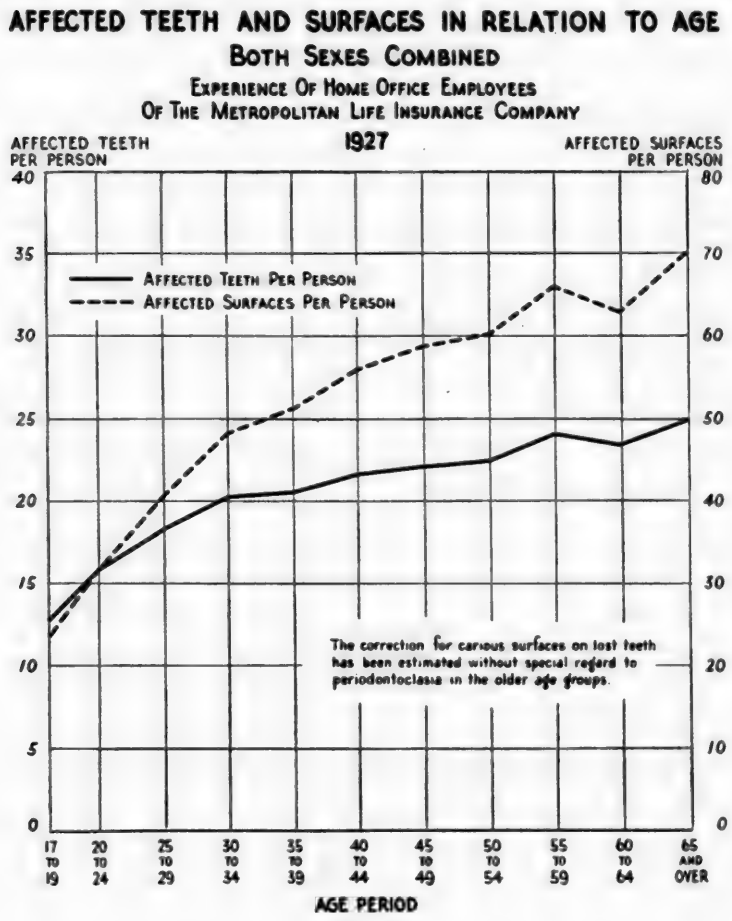
AFFECTED TEETH AND SURFACES IN RELATION TO AGE
BOTH SEXES COMBINED
EXPERIENCE OF HOME OFFICE EMPLOYEES
OF THE METROPOLITAN LIFE INSURANCE COMPANY
1927
AFFECTED TEETH PER PERSON
AFFECTED SURFACES PER PERSON
AFFECTED TEETH PER PERSON
AFFECTED SURFACES PER PERSON
The correction for carious surfaces on lost teeth has been estimated without special regard to periodontoclasia in the older age groups.
AGE PERIOD

FIG. 2

value is the one adopted by Bödecker (8) in the calculation of his caries index, and is based on the gross results seen in a group of 100 extracted teeth from patients of all ages. The value 3 is intended to

measure the carious involvement only of teeth lost by the caries process itself and not by periodontoclasia. Hence, use of this value for the conversion factor introduces an error in our estimate of caries incidence by surfaces; a corresponding error exists also in the data on incidence by teeth. However, since no adequate determination of the exact figure has been made, and it is not even known at what age level pyorrhetic teeth begin to be lost in numbers large enough to be significant statistically, we have not attempted to differentiate between caries and periodontoclasia in this respect. This fact should be borne in mind in interpreting our values for all the age groups above that of perhaps 40–44.

VARIATIONS IN CARIES INCIDENCE WITH AGE

The results of these computations are presented in the accompanying graphs. Data for caries incidence, expressed in terms of surfaces per person, for various ages but without regard to sex is considered first (*fig. 2*). It will be noted that the curve showing the course of the number of affected surfaces per person rises very rapidly for a short distance (corresponding to a rate of about 1.75 surfaces per person per year of age) but at about age 34 it bends over, indicating a progressive slowing down of the rate of development of new lesions with advancing age. During the age period beyond 34 years, the average rate of formation of decay is about 0.5 of a surface per person per year of age,[3] representing a gross reduction of about 70 per cent from the value at the earlier ages.

The second curve (solid line) in *fig. 2*, showing the course of the number of affected teeth per person, represents the data on the same individuals as does the first curve (broken line) but measured in terms of carious teeth per person instead of carious surfaces. The general shape of this curve is essentially the same as that obtained in terms of carious surfaces and offers ample confirmation of the facts, already indicated by the graph in terms of surfaces, that the process of decay

[3] The above assumes that the differences between successive age groups represent solely the effect of advancing age. But it must be remembered that the older persons in the sample have lived through an earlier calendar period, and therefore in a somewhat different environment with different dietary habits, different dental attention, or whatever other external factors influence the rate of decay.

continues right on into advanced age, although the rate of development of new lesions shows a progressive diminution throughout. In neither case is there any evidence of a strikingly abrupt change in curvature of the graphs, as might be expected from the clinical impressions reported. There can be no doubt of a definite, albeit gradual, change in curvature, which is most pronounced in the interval 25 to 35 years. Also, if we were able to make allowance for the unreliability of the factor used to convert the number of missing and crowned teeth to the number of carious surfaces—particularly as this affects the number beyond age 40—this change in slope would perhaps be a little more pronounced than it is in the present study. It must be concluded, therefore, that a definite reduction in the rate of development of dental decay does occur beyond the age of 25, but the decrease is considerably more gradual than one might anticipate from the opinions cited in the introduction. Furthermore—and this is of even more consequence—the reduction occurs so far above age 20 that it cannot fairly be described as specifically associated with the passage of adolescence.

One factor which our study does not properly evaluate is the difference between new caries occurring on smooth normal surfaces and recurrent decay occurring around and under old fillings where rough margins may exist, or where a number of conditions contribute to alter the normal mechanical functioning of the teeth and peridental membranes. It is obvious that such recurrences are much more common in advancing age than in youth, since they depend more upon the number of previous fillings than upon actual susceptibility. Other such factors are the extent to which both pyorrhea and senile decay are included in our evaluation of the caries incidence—factors which operate exclusively in the upper age ranges. Further study along these lines is in order.

Be this as it may, the process of decay is measurably active even at age 50, although the rate of decay, that is, the rate at which new lesions are added to the existing teeth, is considerably less at that age than at age 25. This tendency is indicated by the decrease in the slope of the curves with advancing age in *fig. 2*. The progressive falling off in the rate of formation of new lesions can not be explained by a progressive calcification of enamel with age, because no such

continuous post-eruptive calcification has ever been demonstrated (Hollander, 10).

DIFFERENTIAL INCIDENCE OF CARIES OF THE TEETH IN THE TWO SEXES

Reports in the literature on the comparative incidence of caries in the two sexes are divergent. Out of six such reports of relatively recent date, four (11, 12, 13, 14) indicate a definitely higher incidence among females than among males, one (15) a higher caries index for males, and two (15, 16) practically no sex difference at all. Only one of these studies (16) included age groups above twenty years.

In view of these discrepancies, our own observations are particularly interesting. The respective curves for our entire group of 3,388 males and 9,365 females, with caries incidence expressed in terms of affected teeth per individual, is shown in *fig. 3*. With the single exception of the age group 60–64 years, the curve for females lies consistently above that for males. Up to age 34, the differences between the sexes are small; in fact, the second and fourth points on the curves appear to be almost coincident. If caries incidence be expressed in terms of affected surfaces per person, as in *fig. 4*, instead of affected teeth, this relation between the two curves remains essentially unaltered. The irregularity in the penultimate age group is obviously caused by the marked break in the curve for females at this position, a discontinuity which we cannot explain at this time.

The very appearance of the two curves strongly suggests to the eye that there is a systematic difference in the caries incidence among males and females, with distinctly more caries among the females. Indeed, at ages 35–39 and beyond, this is so apparent that it would seem unnecessary to apply any refined test positively to establish this relation as a real systematic phenomenon, rather than one due simply to such variations as would naturally occur in sampling. At the lower ages, up to 30–34, one may feel doubt on this point until a special test is applied. Such a statistical test for the significance of the difference observed has been applied to the data in which caries was measured in terms of affected teeth per person (Table II). It will at once be seen that the difference in the incidence of caries among males and females is clearly significant in all age groups except

30–34 and 60–64. As to the last-mentioned, the lack of significance may here be entirely disregarded. The sample is too small, and this is the only point on the curve for females which is obviously out of

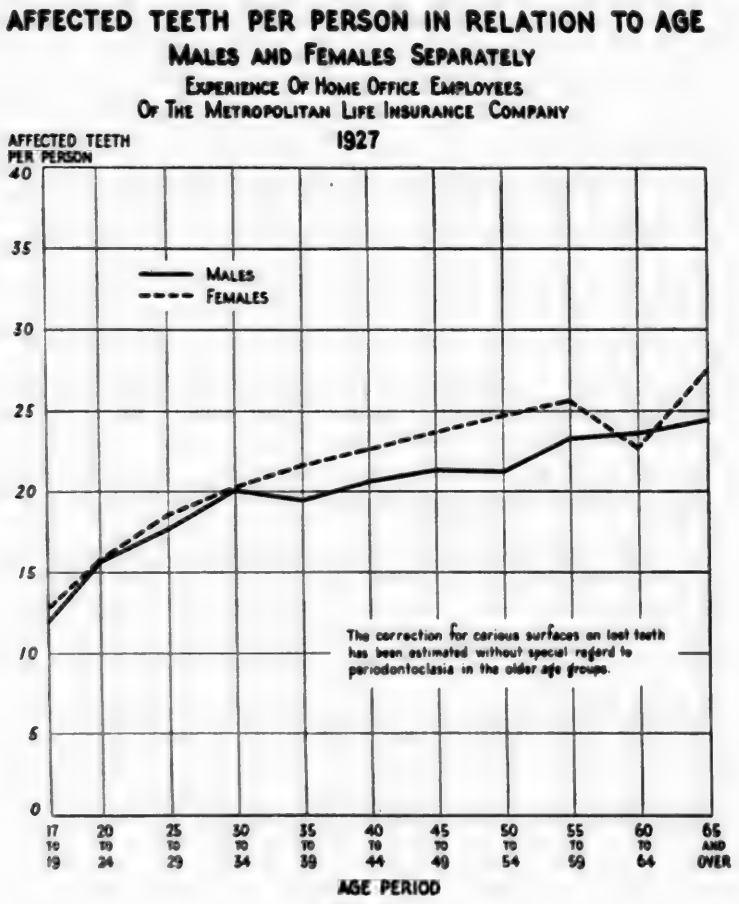

FIG. 3

line. As regards the 30–34 year group it should be remarked that this case is just on the borderline of what by a somewhat arbitrary rule is regarded as the limit of significance. The custom is to regard as significant the difference between two quantities determined from

outwardly similar samples when that difference is more than twice its standard error. In this case the difference in the proportion of affected teeth between males and females is .01232, and its standard

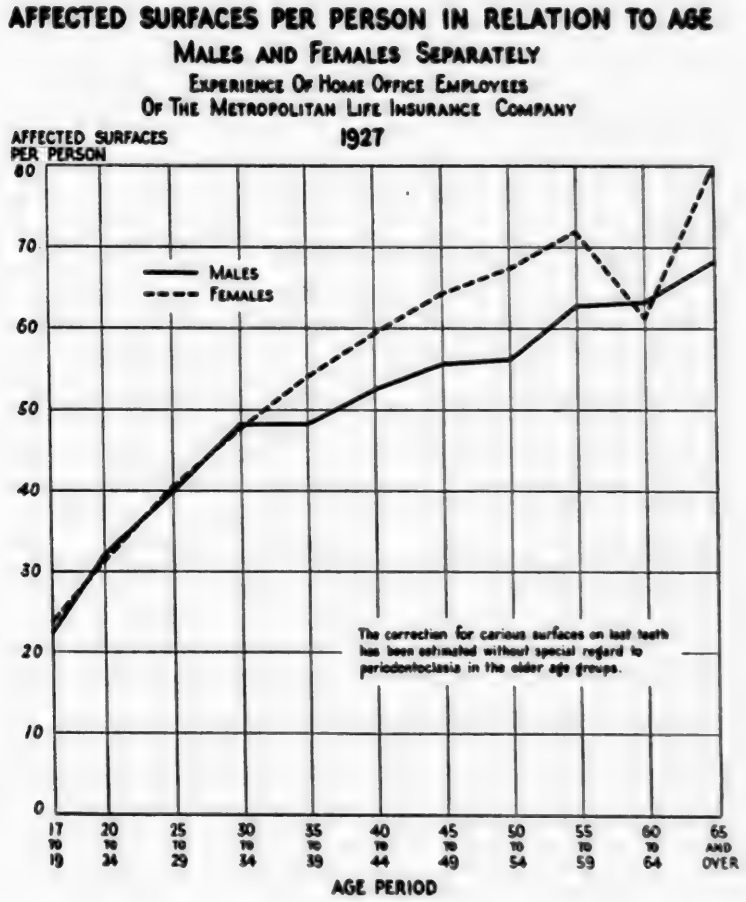

FIG. 4

error is .00622. It will be seen here that the difference comes very near, indeed, being twice its standard error; furthermore, in weighing this evidence, it is entirely proper to take into account the corroborating evidence of all other age groups for which adequate samples

TABLE II

Ratio of affected teeth to total erupted teeth as observed among home office employees of the Metropolitan Life Insurance Company, classified by age and sex, 1927

AGE AND SEX	TOTAL NUMBER OF ERUPTED TEETH (n)	NUMBER OF TEETH AFFECTED (a)	DIFFERENCE IN PROPORTION (q) BETWEEN FEMALES AND MALES (d)	STANDARD ERROR OF DIFFERENCE BETWEEN FEMALES AND MALES IN PROPORTIONS OF TEETH AFFECTED (σ_d)	SIGNIFICANCES OF DIFFERENCE (d)
7–19 M.	16516	6763	.03073	.00411	Yes
F.	109535	48218			
20–24 M.	26738	13756	.00947	.00342	Yes
F.	107381	56261			
25–29 M.	13985	7892	.03144	.00515	Yes
F.	26677	15893			
30–34 M.	11351	7189	.01232	.00622	No
F.	12570	8116			
35–39 M.	10071	6211	.06286	.00697	Yes
F.	8676	5896			
40–44 M.	6966	4520	.06152	.00794	Yes
F.	6778	4815			
45–49 M.	7920	5271	.07733	.00847	Yes
F.	4375	3250			
50–54 M.	5264	3495	.10656	.01036	Yes
F.	2719	2095			
55–59 M.	3418	2488	.07493	.01238	Yes
F.	1659	1332			
60–64 M.	1568	1156	−.02548	.02294	No
F.	510	363			
65 + M.	1184	905	.09502	.02796	Yes
F.	192	165			

In applying the test, the following relations were employed:

$q = \frac{a}{n}$ = the proportion of teeth affected.

$\sigma_q = \sqrt{\frac{q(1-q)}{n}}$ = the standard error of q.

$d = q_f - q_m$ = the difference between q for females and q for males.

$\sigma_d = \sqrt{(\sigma_{q_f})^2 + (\sigma_{q_m})^2}$ = the standard error of d.

A d-value is considered statistically significant when it is more than twice its standard error.

were available. For these reasons, it may be concluded that here also the observed difference is significant.

In order to corroborate the foregoing, we performed a similar comparison of the graphs for males and females, wherein caries incidence was measured by affected surfaces (*fig. 4*), and applied the significance test to these new differences. The results are essentially the same as those obtained with the data in terms of affected teeth (*fig. 3*), except at one point—that corresponding to age group 20–24. In this case, the incidence of decay in terms of affected surfaces is slightly higher for males than for females, although it is slightly lower for males when measured in terms of affected teeth. This reversal, occurring as it does in only one age group and apparently not in accord with the general trend toward sex difference shown by the rest of the data, is puzzling and must be accepted with reservation. One possible explanation, however, may be gleaned from the fact that the number of males within the age group 20–24 years is decidedly greater than that for the age group 17–19 years, whereas the numbers of females within these two age groups are not appreciably different. The implication is that the males within the age group 20–24 years had been employed by the Metropolitan Life Insurance Company on the average more recently than the females within the same range. In general, it may be expected that such newly employed individuals had received less dental attention than those who had been in the service of the Company for a year or more and who had consequently been subject to the careful dental scrutiny assured by this employment. It may be concluded, therefore, that the differential incidence of caries in the two sexes is independent of whether it be expressed in terms of affected teeth or affected surfaces, with the one exception noted.

The net conclusion, thus, is that whenever the sample was adequate there was found at every age a significant difference, with the females showing a higher caries incidence than the males. Up to age 34, the differences were only slight, beyond this age the differences were quite marked. Thus our own results are in essential agreement with the observations of other investigators cited above, with the exception of the findings of Day and Sedwick for 13 year old children of European origin and of Driak for patients of all ages. Regarding the reasons

for the sudden divergence of the curves above age 34, that is, the sudden increase in the difference between the male and female incidence of caries in these higher ages, we are as yet unable to offer any satisfactory explanation.

CONCLUSIONS

(1) The initiation of the new lesions of dental caries must continue past childhood and adolescence at least until middle life, at a slowly and progressively decreasing rate. Although the rate of new lesions diminishes gradually, this diminution appears to be more marked during the age period from 25 to 35 years than during any other period of like duration and not apparently linked to the process of adolescence. This reduction in rate of development of new lesions can be ascribed in part but not entirely to a decrease in the residual number of unaffected, decayable teeth (or tooth surfaces) which are present in the mouth at any particular age.

(2) Throughout almost the entire age range from 17 to 65 the incidence of caries is significantly higher among women than among men, the difference being very slight below the age of 34 years, but increasingly marked above that age.

(3) When analyzed for differences in incidence of caries, a group of persons which represents an extensive cross section of the population and which has been gathered together for reasons other than dental disease, gives a different result from a group of individuals of similar social and economic status which has been studied at the time of dental treatment. The former group is to be considered preferable for statistical purposes concerned with the community as a whole, but either group can be used for the investigation of such problems as the relative incidence of different types of decay.

(4) Our statistical analysis of caries incidence for the whole mouth in terms of decayed surfaces per person yielded essentially the same conclusions as did an analysis in terms of decayed teeth per person, and therefore the two criteria of caries incidence appear to be almost equally satisfactory for any study which, like the present one, includes a sufficiently large number of persons. Decayed surfaces, however, offer the more sensitive criterion of the two.

(5) The technique employed in this study for evaluating caries

incidence involves several specific sources of error which must be borne in mind for future investigations. Senile decay, due to gingival recession, has been included in the caries count; the degree of influence of this inclusion is uncertain and as yet we are not prepared to express a definite opinion on it. Also, it has been assumed that all the missing teeth were lost because of decay. This is obviously an erroneous assumption, since some of them which were lost because of pyorrhea were also affected by caries. To correct this latter error, it will be necessary to reevaluate the factor 3, by which the number of missing teeth was multiplied in order to convert it to number of carious surfaces, giving special attention to the variation of this figure with age and sex. In general, any change in our curves resulting from the use of a revised value should take the form of a somewhat more marked difference in the rate of development of new caries lesions between the earlier and the later age groups—particularly beyond 40 years—because of a lowering of the number of caries lesions estimated from the number of missing teeth. What effect this will have on the distribution by sexes, it is impossible to anticipate.

We wish to acknowledge most gratefully the assistance of Dr. Alfred J. Lotka, Assistant Statistician of the Metropolitan Life Insurance Company, and Mr. M. Spiegelman of the statistical staff of the same Company, not only in the completion of the significance tests reported, but also in helping to outline our entire approach to the statistical analysis of our data. To Mr. M. Goldstein, in charge of the mechanical operation of the Company's Statistical Bureau, we are obliged for the planning and supervision of the mechanical tabulating and sorting procedures involved. Dr. Charles F. Bödecker and Dr. Theodor Rosebury have read this manuscript with great care and we are also grateful to them for their several valuable criticisms. Above all, we are indebted to Dr. Thaddeus P. Hyatt for his original work in collecting and assembling the Metropolitan data.

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VARIATIONS IN THE ORAL FLORA FROM TIME TO TIME[1]

MAYNARD K. HINE, D.D.S., M.S.[2] AND BASIL G. BIBBY, B.D.S., PH.D.

From the Division of Dental Research, School of Medicine and Dentistry, The University of Rochester, Rochester, New York

The widely divergent percentages of organisms found in the flora of different mouths (Bibby, 1938b) and in different parts of the same mouth (Bibby, 1938c) suggest that variations occur in the oral flora from time to time. This possibility has never been thoroughly studied. Such variations are indicated in two reports. In one, Goadby (1910) claims to have changed the basic nature of the oral flora by means of a strict hygienic and dietary regime. In the other, Howitt and Fleming (1930) note differences in the oral flora on different days. The following work was carried out to determine the constancy of the numbers of the different groups of microörganisms in the flora of certain parts of the mouth.

METHODS AND MATERIALS

Specimens for examination were collected from distinctive areas in mouths at the same time on successive days. Use of the same finger rests and dental instruments made a reproducible technique possible. Before collection, a standard procedure of mouth rinsing with sterile saline, and isolation of the test areas was followed (Bibby, 1938b).

Daily examinations were made for 18 days of mouth washings and material from a gingivo-mesial cavity in an upper premolar. Other studies were made on 16 smears taken during 18 days from the distal surface of the last lower molar and from a caries-free occlusal fissure of an upper premolar. The mouths used for these studies were those of the authors, neither of which have any gross pathological defects. Fortnightly studies were made over 12 weeks of material from two areas in healthy mouths of 10 adults. One series of smears was made from the distal surfaces of the lower third molars, 7 of which were covered by gingival tissue flaps. Another series of smears was made from the bucco-gingival surfaces of the upper right first molars of 8 patients and in 2 instances from the corresponding surfaces of the upper right second premolar or the lower right second premolar.

The estimates of the percentage occurrence of different microorganisms in the flora

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[2] Now at University of Illinois, College of Dentistry.

were made by a direct counting method, (Bibby, 1938a). The classifications of organisms previously used were modified on the basis of newer knowledge.

FINDINGS

The percentages of the various groups of microörganisms found in the daily smears are summarized in Table I and in *fig. 1* the actual percentages found in smears from the lower molars are set out graphically. Tables II and III show the average and maximum and minimum percentages of the different bacterial groups encountered in the fortnightly smears from different mouths.

TABLE I

Percentage occurrence of groups of organisms in daily smears

SOURCE	NO. OF SMEARS	PERCENTAGES OF TOTAL ORGANISMS COUNTED							
		Cocci		Bacilli		Filaments		Fusiformis
		Gram +	Gram −	Gram +	Gram −	Gram +	Gram −	
Posterior lower molar	18	Range 41.3–55.6	18.1–29.5	0–8.5	1.5–10.0	0.5–11.4	2.6–10.0	5.1–10.1
		Av. 49.0	23.9	3.3	5.1	5.4	8.1	8.0
Cavity	18	Range 40.3–50.0	20.0–30.2	0.5–6.6	4.0–12.3	0.8–7.3	2.0–10.2	6.7–14.3
		Av. 44.1	23.4	3.5	8.4	3.9	6.7	10.1
Mouth washings	16	Range 38.4–65.4	18.7–46.8	0–2.5	0–6.3	0–5.2	1.2–6.6	1.4–9.3
		Av. 54.7	30.1	0.5	2.8	2.6	4.3	5.0
Occlusal fissure	16	Range 32.6–58.8	19.2–52.1	0–1.8	2.9–10.9	0–8.4	3.6–12.9	0–8.3
		Av. 50.3	30.2	0.6	6.0	3.1	6.5	3.3

The results reveal little constancy in the percentages of the various groups from time to time. The extent of the variations are not the same for all groups of organisms, nor do they follow the same pattern in the smears from different parts of the same mouth. Only a few features are worth comment.

The total percentages of cocci found in the daily smears were fairly constant, those of the Gram-positive forms being consistently higher than the Gram-negative. This was especially marked in the material from the lower molars where the percentages of Gram-positive cocci were consistently more than 16 per cent higher than those of the Gram-negative cocci. There was less uniformity in the distribution of the other types of organism. Filamentous groups generally maintained a higher level than the fusiform or bacillary types. This

difference was least marked in the series of smears from the cavity where the percentages of fusiform bacilli and Gram-negative bacilli approximated those of the filaments. The Gram-positive bacilli were generally present in the smallest numbers, most exceptions being found in the series obtained from the lower molars.

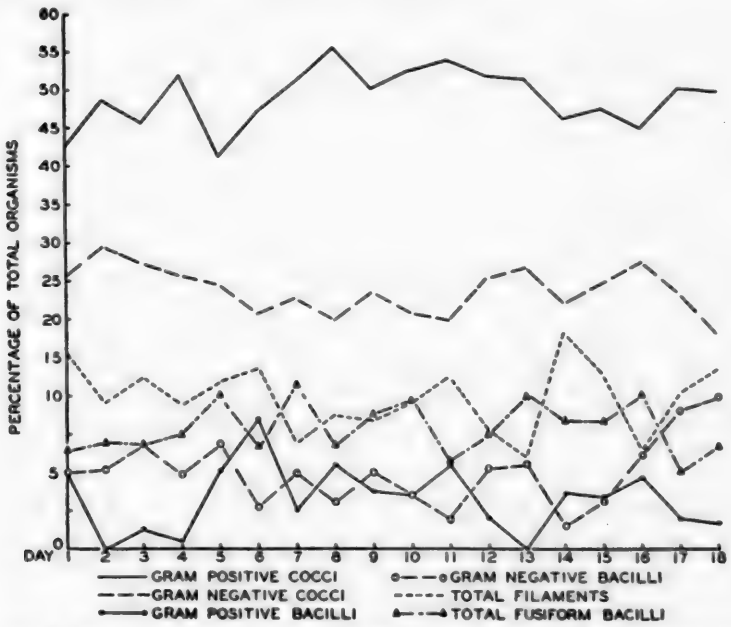

Fig. 1. Percentages of different organisms on repeated smears from posterior surface of lower molar.

The variations in the fortnightly smears were comparable to those in the daily series. Marked dissimilarities appeared between the flora of the various mouths and lesser differences between the 2 areas from the same mouth. In 2 mouths the percentages of cocci in the 2 areas gave completely parallel variations from day to day. However, when the changes in the percentages of all types of organisms in the 2 sites in these mouths were compared they were in the same direction in only 56 per cent of the instances. In some mouths the numbers of particular types of organisms showed general trends upward or

downward during the period of study. In other mouths certain groups of organisms maintained consistently high or low levels.

DISCUSSION

The results presented indicate that over short periods of time there is some tendency towards uniformity in the percentages of various

TABLE II

Percentage occurrence of groups of organisms in fortnightly smears from disto-gingival surface of lower molars

PATIENT		COCCI		BACILLI		LEPT.	VIBRIOS	FUSIFORMIS		
		Gram +	Gram −	Gram +	Gram −			Nucl.	Polym.	Dent.
A	Range	51.8–68.7	13.2–28.8	0–6.1	2.0–8.2	0–1.3	0–3.7	1.0–5.5	0–5.5	1.2–8.9
	Av.	63.5	19.8	1.8	4.8	0.2	1.6	2.8	2.2	3.3
B	Range	24.8–48.2	20.0–37.2	0–3.3	0.6–11.6	0.5–14.2	0–4.5	5.8–15.7	4.2–9.3	0–7.8
	Av.	38.3	26.0	1.1	7.3	5.0	1.3	10.5	7.5	3.0
C	Range	38.4–55.1	22.4–29.6	1.6–3.6	3.2–10.6	0–1.8	0–2.0	2.5–10.6	2.4–7.8	2.0–19.7
	Av.	44.6	25.9	2.1	6.7	0.7	1.0	5.8	5.8	7.4
D	Range	38.3–68.7	17.4–44.9	0–7.5	1.5–3.9	0	0–2.9	0–8.0	2.2–10.2	0–3.3
	Av.	57.1	27.1	2.0	2.7	0	0.7	3.7	5.8	0.9
E	Range	35.9–57.1	21.1–30.6	0–5.2	4.2–6.6	0–5.9	0–7.9	7.6–12.6	1.5–6.7	0–5.2
	Av.	44.9	26.1	1.6	5.3	2.0	2.9	10.4	4.2	2.6
F	Range	35.2–48.5	17.6–32.2	0–5.6	5.9–10.6	0–4.8	0–2.4	4.1–16.2	3.4–9.0	0.7–6.2
	Av.	41.4	24.7	2.3	7.6	1.3	1.5	11.3	6.2	3.7
G	Range	28.2–67.4	16.7–44.4	0–4.2	1.3–6.5	0–13.2	0–4.3	1.8–8.5	0.6–11.6	0–5.4
	Av.	50.1	25.0	2.4	3.8	3.1	2.0	5.5	6.1	2.0
H	Range	30.3–43.6	12.7–30.7	2.3–3.8	4.5–9.8	0–5.8	0–18.0	8.0–23.1	1.7–18.0	0–7.0
	Av.	38.0	20.8	2.8	6.3	2.1	5.7	13.7	6.7	3.9
I	Range	31.5–53.9	13.8–34.5	0–5.0	6.5–9.1	1.1–11.3	0–11.2	1.7–6.4	2.2–7.2	1.0–5.0
	Av.	41.2	26.7	2.5	7.5	4.5	4.2	4.7	5.0	3.7
J	Range	32.2–37.9	15.5–23.1	0–4.0	1.3–8.8	0–10.9	0–1.6	10.3–20.0	6.0–14.7	6.0–9.5
	Av.	35.3	20.1	1.7	5.5	3.2	0.5	15.3	10.4	8.0

types of organisms occurring in different parts of the mouth. Although the differences in the averages between some mouths are, of themselves, to be sufficiently great, to be statistically significant by the counting method employed, the variations in the percentages from time to time invalidate the significance of such differences. However, the results can be taken to indicate the existence of more

or less permanent differences between the flora of similar areas in different mouths.

On the basis of these findings it is at once apparent that no great reproducibility can be expected in smears taken on different days from a single area in a mouth. The findings further indicate that most of

TABLE III

Percentage occurrence of groups of organisms in fortnightly smears from buccal surfaces of upper molars

PATIENT		COCCI		BACILLI		LEPT.	VIBRIOS	FUSIFORMIS		
		Gram +	Gram −	Gram +	Gram −			Nucl.	Polym.	Dent.
A	Range	54.7–93.1	6.9–30.1	0–4.6	0–4.6	0	0	0–6.5	0–5.8	0–3.2
	Av.	70.7	19.8	2.0	4.6	0	0	3.2	2.0	1.3
B*	Range	38.3–72.3	15.3–31.3	0–3.5	0.7–11.7	0–4.0	0–1.6	5.1–15.0	2.2–13.4	0–2.9
	Av.	52.3	21.4	1.6	4.4	2.4	0.4	9.3	7.3	1.0
C	Range	28.6–70.1	15.7–26.9	2.0–13.7	0–23.6	0–2.0	0–3.0	1.8–5.0	0.6–7.2	0–3.7
	Av.	55.6	21.5	5.6	7.2	0.3	0.9	2.8	4.9	1.2
D	Range	36.2–51.3	20.7–31.3	1.2–2.6	5.3–12.4	0	0–1.0	4.3–22.1	4.6–9.0	0–6.9
	Av.	46.4	25.5	2.3	7.6	0	0.3	9.5	5.5	2.9
E	Range	39.5–85.6	6.3–23.5	1.2–3.1	0–10.5	0–0.6	0–6.0	0–12.0	3.8–8.7	0–4.0
	Av.	63.3	17.0	2.2	2.8	0.1	1.1	6.2	5.2	2.1
F	Range	36.1–67.9	19.0–24.5	0–8.5	0.8–9.8	0–2.6	0–3.0	3.7–14.6	2.9–7.7	0–2.9
	Av.	49.3	21.6	3.3	7.4	0.5	0.7	9.9	5.5	1.8
G	Range	32.8–58.3	8.2–31.5	0–5.9	0.6–8.2	0–1.6	0–1.1	1.6–32.9	2.8–9.1	2.3–19.5
	Av.	44.9	22.0	2.3	5.3	0.6	0.3	12.4	5.6	6.6
H†	Range	34.1–60.5	12.7–35.1	0–6.0	2.2–8.5	0–4.6	0–2.0	4.0–9.0	2.6–6.6	3.1–7.5
	Av.	50.2	24.6	2.8	4.9	1.2	0.7	6.5	4.4	4.7
I	Range	55.5–84.5	12.1–28.2	0–3.2	1.7–3.6	0	0	0–4.3	0–7.5	0–2.1
	Av.	66.6	24.2	1.3	2.8	0	0	1.8	2.8	0.5
J	Range	24.4–67.9	13.7–31.2	0–5.9	1.4–7.9	0–5.4	0–1.3	0–21.6	1.3–8.7	0–21.6
	Av.	52.1	22.6	1.2	4.0	1.6	0.2	8.2	5.6	4.5

* Smears taken from second upper premolar.
† Smears taken from first lower premolar.

the inconsistent results observed when an attempt was being made to establish the characteristics of the flora in different parts of the mouth (Bibby, 1938c) can be accounted for on the basis of the daily variations demonstrated here. However, some of the wider variations encountered at that time must be considered as manifestations of

definitely aberrant flora. Before such unusual flora can be definitely recognized it will be necessary to establish the variations likely to occur from time to time in a number of different mouths. Similarly to establish the existence of an unusual or potentially pathogenic flora, it would be necessary to make a number of separate examinations at suitable intervals of time.

These observations emphasize the difficulties of determining the influences which modify the oral flora or of establishing differences in the flora of different mouths. The value of these findings rests in the demonstrations of the types of variation which occur in some of the bacterial constituents of the oral flora.

SUMMARY

Repeated smears of specimens taken from the same areas about the teeth were examined to determine whether variations occurred from time to time in the bacterial flora of given areas.

It was shown that considerable variations occur from time to time. There were some indications that one or another group of microorganisms maintained a relatively constant level in some mouths. None of these differences was large or constant enough to be statistically significant.

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A STUDY OF TEMPORARY FILLINGS AS HERMETIC SEALING AGENTS

LOUIS I. GROSSMAN, D.D.S., Dr. med. dent.

Philadelphia, Pa.

The adequacy or inadequacy of temporary filling materials for sealing root canal dressings has been little studied. It has generally been assumed that any commercially marketed plastic or cementitious material put up for the purpose can be safely used as a temporary filling and will form a hermetic seal. While a bacteria-tight seal may not be required under certain circumstances, the necessity for having an hermetic seal during root canal treatment is obvious. For this reason, a study was made of the permeability to dyes, to saliva, and to test organisms, of the materials more commonly used as temporary fillings.

The materials examined were temporary stopping, base plate gutta percha, oxyphosphate of zinc cements, "Pro Tem," and zinc oxide-eugenol cement. Combinations of these materials were also tried, such as a double layer of gutta percha, and a layer of oxyphosphate cement over base plate gutta percha.

PROCEDURE

After trying several methods, including extracted teeth and Ivorine blocks, glass capillary tubes were found to give the most uniform results and enabled direct visual examination. For this purpose, thin glass tubing having an inside diameter of about 3 mm. was cut into 15 cm. lengths. The ends were left unpolished. The inner surface, where the filling was to be placed, was roughened with a coarse carborundum stone to imitate the roughness of a dentinal surface. Each tube was then filled at one end with the test filling material to a depth of about 2 or 3 mm. The rest of the tube was packed with a cotton wick in the form of a loosely woven string, thick enough to fill the inside of the tube. (*fig. 1*) Tubes prepared in this manner were suspended in an aqueous solution of dyestuff, the filled end of the tube being submerged, the unfilled, open end extending above the level of the dye. The dye generally used was methylene blue. Substitution of gentian violet, carbol fuchsin or Sudan III in those cases where tried, did not alter the outcome of the tests. Each dye test was repeated a minimum of 10 times and a maximum of 24 times. The bacteriologic tests, to be described later, were run only in duplicate.

RESULTS

Of 10 sealing agents, or combination of sealing agents, repeatedly tested in this manner, 6 definitely leaked, 1 gave variable results, and 3 did not leak. It should be noted here that although gutta percha and the oxyphosphate cements showed evidence of leakage, such

Dye alone

TEMPORARY FILLING	LEAKAGE	NO LEAKAGE
Temporary stopping	*	
Base plate gutta percha	*	
Gutta percha and oxyphosphate cement #1	*	
Oxyphosphate cement #1	*	
Oxyphosphate cement #2	*	
Zinc oxide and eugenol cement		*
Pro Tem cement	?	
Double layer of gutta percha	*	
Modified zinc oxide-eugenol cement #1		*
Modified zinc oxide-eugenol cement #2		*

leakage must have taken place at the periphery only, since pellets of gutta percha and of cement suspended in a dye solution for several days became discolored upon the surface only and showed no significant penetration by the dye.

In another set of tests,[1] saliva colored with methylene blue was used as the test medium because it more closely approximated conditions prevailing in the mouth. The results were uniformly better, so far as preventing leakage is concerned. While 4 filling materials gave somewhat variable results, 6 filling materials definitely prevented leakage. On the assumption that the viscosity of saliva helped to prevent leakage, this test was repeated using a solution of gum acacia in place of saliva. The results were similar, suggesting that viscosity is a factor in preventing leakage. It is conceivable that the molecules of protein present in saliva, being larger than those of the dyestuff, have more difficulty in getting through a temporary filling which is permeable to an aqueous dye solution. As a further check upon this assumption, capillary tubes prepared in the manner already described

[1] Suggested by Dr. J. L. T. Appleton.

which had previously been suspended in saliva, were removed, the outside surfaces were washed, and the tubes were suspended again in an aqueous solution of methylene blue. Some of those that had not shown leakage while suspended in saliva, leaked and became discolored by the dye.

Saliva plus dye

TEMPORARY FILLING	LEAKAGE	NO LEAKAGE
Temporary stopping		*
Base plate gutta percha	?	
Gutta percha and oxyphosphate cement #1		*
Oxyphosphate cement #1	?	
Oxyphosphate cement #2	?	
Zinc oxide and eugenol cement		*
Pro Tem cement		*
Double layer of gutta percha	?	
Modified zinc oxide-eugenol cement #1		*
Modified zinc oxide-eugenol cement #2		*

Bacteriologic tests, using *Bacillus prodigiosus* and mouth organisms as a means of detecting leakage were also tried. The results previously obtained with dyes and with saliva were substantially confirmed by these tests. Due to technical difficulties involved in the placing of temporary filling materials in the capillary tubes without causing contamination, these tests were run only in duplicate, especially since they seemed only to confirm previous tests.

DISCUSSION

Two results which had not been anticipated were observed: (1) when capillary tubes were filled with oxyphosphate of zinc cement at one end, as previously described, and the filled ends were lowered in a dye solution or in tap water, the dye or water rose in the capillary tubes, indicating leakage of the fillings. (2) Zinc oxide-eugenol fillings, which the author used to look upon with misgivings, were found to be leak-proof, without exception, in all tests. This is probably due to the fact that the filling material expands on contact with moisture. Since deformation of a zinc oxide-eugenol filling may easily occur because of its prolonged setting time, with consequent contamination,

the setting time of such cements may be hastened by the addition of clear rosin and benzoic acid, as in the following formula:

Powder

Zinc oxide (Reagent)	70
W. W. Rosin	20
Zinc stearate	8
Benzoic acid	2

Liquid

Eugenol	88
Olive oil	12

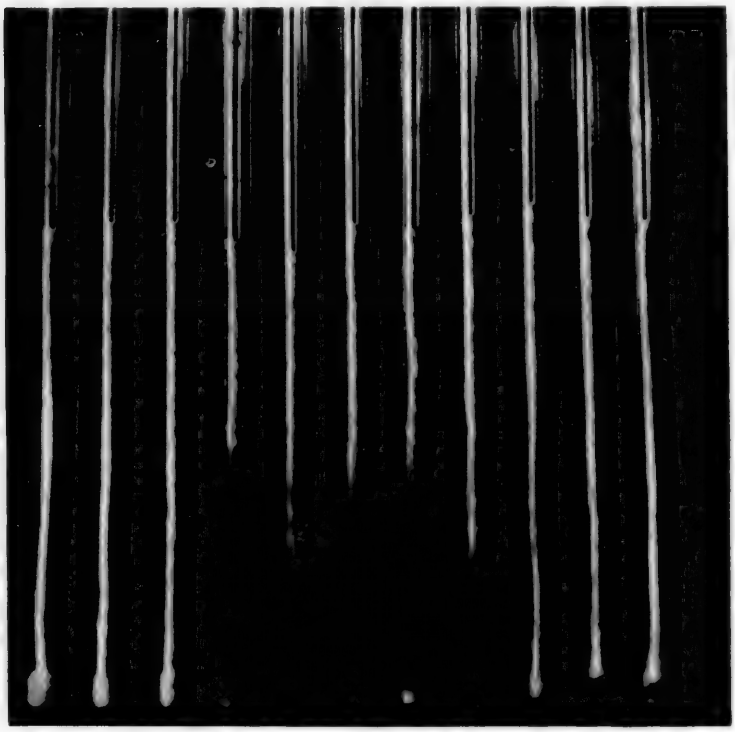


FIG. 1. Method of testing peripheral permeability of temporary filling materials. Capillary tubes filled for 2 or 3 mm. at bottom with test materials. Tubes then suspended in dye solution or dye-saliva solution. Staining of cotton wicks (4–8 inc.) evidence of leakage.

It might be added here that while an hermetic seal is the prime requisite of a temporary filling material used as a dressing seal, other properties such as strength, adaptability, etc. should be considered. The objection to oxyphosphate cement, aside from leakage, has been that after grinding to occlusion, the filling has often become so thin as to cave in during mastication. Whenever possible, a double seal should therefore be used, i.e., an inner seal of gutta percha and an outer seal of cement. Should the cement surface break, the root canal is still protected by the inner seal of gutta percha. A thin layer of gutta percha covered with a quick setting zinc oxide-eugenol cement approaches in many ways the ideal temporary filling. This type of filling not only forms an hermetic seal but prevents chips of cement from falling into the root canal.

SUMMARY AND CONCLUSIONS

(1) Ten temporary filling agents, commonly used for sealing root canal dressings, were tested for hermetic properties against dye solutions, saliva colored with a dye, and against bacteria. (2) Temporary stopping, base plate gutta percha, oxyphosphate cement, Pro Tem, either alone or in various combinations are not peripherally impervious to dye solutions or to suspensions of bacteria. In contact with saliva, however, they are less apt to leak. (3) Cements having a zinc oxide-eugenol base were found to be hermetic under all conditions of the tests. (4) As a group, the zinc oxide-eugenol cements appeared to be the best, oxyphosphate cements the worst, while gutta percha fillings occupied an intermediate position in preventing peripheral leakage. (5) In a study of this type, variables affecting technic are apt to be introduced. While the data presented here are not so definite as to warrant dogmatic deductions, they are strongly suggestive of the conclusions drawn.

EXPERIMENTS DEALING WITH FACTORS INFLUENCING THE SHEDDING OF DECIDUOUS TEETH

Part I. Radiographic Study

HARRY H. SHAPIRO and WILLIAM M. ROGERS

Department of Anatomy, Columbia University, N. Y.

INTRODUCTION

Shedding of the deciduous teeth has been attributed to pressure caused by the erupting permanent teeth. If this were always true, it would be logical to expect that when there is a failure of the permanent tooth germ to develop, resorption would not occur. In cases of congenital loss of permanent tooth germs, it is often noted that the root of the deciduous tooth is resorbed, despite the fact that there is no permanent tooth germ (1) present which might cause pressure.

Since there are no experimental data which have come to our attention regarding the accepted theory of resorption, experimental means were devised for testing the validity of the theory of pressure.

Permanent tooth germs of cats were removed at several stages during their development, before the onset of resorption of the deciduous root. This form was selected because the curve of the developing dentition is similar to that of man, and also because it has been shown previously that the cat is especially suited for experimental work on dentition (2, 3). The permanent canine tooth was selected for study because of its accessibility and large size. The germ was removed on one side of the mandible, and the unoperated side served as a control.

DESCRIPTION OF OPERATION

Ether anesthesia was used. The lower jaw and neck were shaved. An incision was made to the right of the median line, beginning at the symphysis and continuing posteriorly about $\frac{3}{4}$ inch, and the skin retracted. The incision was then extended through the fascia and periosteum, along the inferior border of the mandible on the right side, exposing the bone. With a surgical bur, a window was cut through the outer alveolar

plate along the inferior border of the mandible just medial and posterior to the apex of the root of the deciduous canine tooth, the position of which had been determined by pre-operative radiographs (*fig. 1*).

The hooked end of a dental explorer was then inserted through the opening in the bone, and the germ of the developing permanent canine tooth was removed. The tooth germs removed at each operation were fixed. The edges of the opening in the cortical bone were then smoothed, the tissue and skin sutured with #5 silk, and post-operative radiographs were taken (*fig. 4*).

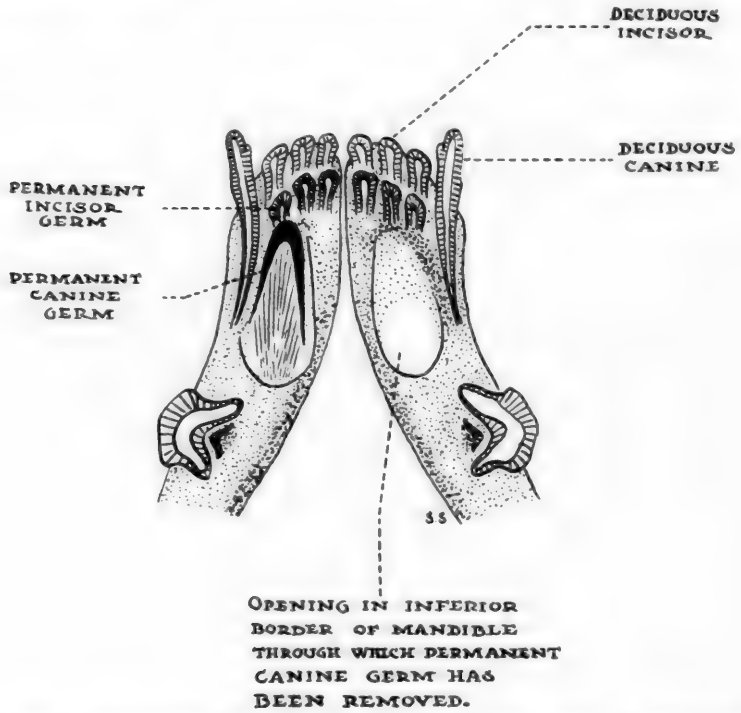

DECIDUOUS INCISOR
DECIDUOUS CANINE
PERMANENT INCISOR GERM
PERMANENT CANINE GERM
OPENING IN INFERIOR BORDER OF MANDIBLE THROUGH WHICH PERMANENT CANINE GERM HAS BEEN REMOVED.

FIG. 1. Diagrammatic representation of x-ray of anterior region of mandible of kitten, indicating area from which permanent canine tooth germ has been removed.

The operation was performed on kittens at two different stages of development as follows: (1) When calcification of the crown of the permanent tooth is first clearly indicated by radiographs (*fig. 2*), and (2), shortly before calcification of the root of the deciduous canine tooth is complete, at a time when the crown of the permanent canine tooth is well along in its development (*figs. 3, 5 and 10*). These two stages were used to determine whether or not the stage of calcification of the developing permanent germ, and the stage of calcification of the temporary root, affected the results obtained from our operative procedures.

OBSERVATIONS

The observations are based on progressive radiographs made at intervals from the time of operation until the animals were sacrificed. This report will consider only those cases which show no injury to the root of the deciduous tooth, thus eliminating any possibility of injury as a factor in the results. A separate study on the relation of injury to the shedding of teeth is being made.

Radiographs made immediately after the operation showed, **1**) complete unilateral absence of the permanent canine germ; **2**) a well defined thin shell of bone, the alveolus, surrounding the space previously occupied by the developing tooth germ; **3**) absence of a small rectangular area of cortical and underlying alveolar bone removed at operation; and **4**) the position, relations, and stage of development of the deciduous teeth and permanent tooth germs (*figs. 1–4*).

FIG. 2. Age 6 weeks

FIG. 3. Age about 15 weeks

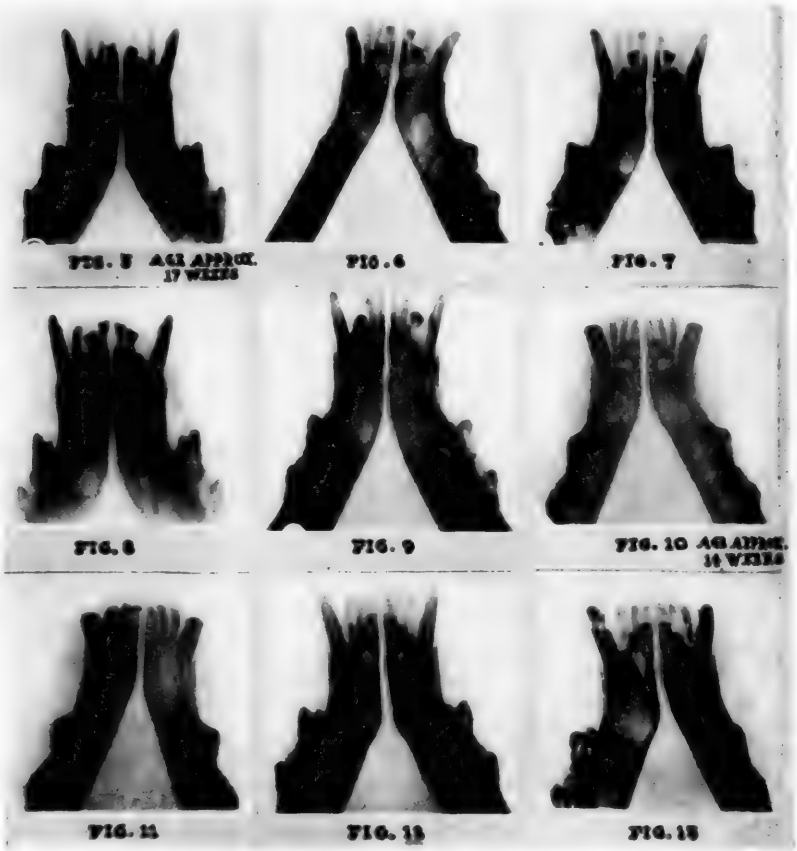
FIG. 4

DESCRIPTION OF CASES

Kitten T—Age approximately 17 weeks, (just before calcification of the deciduous canine root is complete). *Fig. 5* is a pre-operative radiograph of the mandible and *fig. 6* a radiograph taken immediately following removal of the permanent tooth germ. *Fig. 7*, (a radiograph taken 5 days following operation), shows some regeneration of bone in the permanent germ cavity, and some remnants of the shell of alveolar bone lining the alveolus, which had previously contained the tooth germ. Resorption of the root of the deciduous canine on the unoperated side, along its medial border, is first indicated at this time. There is no evidence of resorption of the corresponding root on the operated side. *Fig. 8*, (14 days following operation), shows that the remnants of the shell of bone lining the permanent tooth germ cavity have disappeared completely, and that bone repair in the region of the operation is fairly complete. At this time, resorption of the deciduous canine root on the operated side is first indicated. On the control or unoperated side, resorption has progressed further. *Fig. 9*, (radiograph on the 23rd day following operation), shows complete bone regeneration on the operated side, and resorption

of the deciduous root, adjacent to the developing tooth, on the unoperated side. The root of the deciduous canine on the operated side also shows resorption, advanced over that shown 9 days previously (*fig. 8*).

Litter 1—Kitten *2—Age 14 weeks (about three weeks before the completion of calcification of the deciduous canine root). *Figs. 10 to 23* are a series of pictures taken,



preoperatively (*fig. 10*), and post-operatively (*fig. 11*), and at progressive intervals through 102 days following operation, until the animal was sacrificed. *Fig. 12*, (a radiograph 20 days after operation), shows the deciduous canines fully calcified. *Fig. 15* (40 days after operation) shows the first evidence of resorption along the medial border of the deciduous canine root, adjacent to the developing permanent canine on the control side. On the

operated side, there is no indication of resorption of the deciduous canine root. *Figs. 16, 17, 18, 19, and 20* (40 to 92 days after operation), show progressive resorption of the deciduous canine root on the control side. *Fig. 19*, (radiograph 75 days after operation), first indicates resorption of the deciduous root on the operated side. This resorption begins at the apex. *Fig. 21*, (92 days following operation), shows that the deciduous

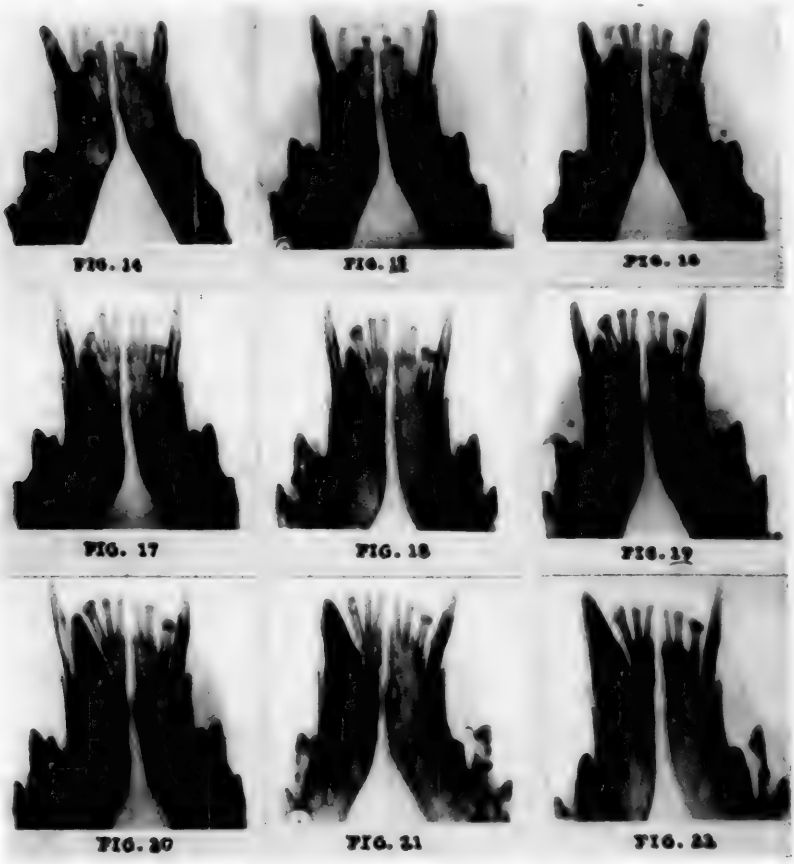


FIG. 16 FIG. 17 FIG. 18

FIG. 19 FIG. 20 FIG. 21

tooth on the control side is shed. *Fig. 22*, (99 days after operation), shows evidence of marked resorption of the deciduous canine root on the operated side, progressing from the apex of the root, within the pulp canal as well as the outer surface surrounded by alveolar bone. *Fig. 23*, (102 days following operation), indicates still more resorption of the deciduous canine root on the operated side progressing in the same manner as described for *fig. 22*.

Litter 1—Kitten #3—Age 14 weeks. *Fig. 24* is a pre-operative radiograph. *Fig. 25* is a postoperative radiograph showing removal of the canine germ. *Fig. 26*, (14 days following operation), shows complete calcification of the deciduous canine roots, and bone regeneration of the entire operated area. *Fig. 27*, (47 days following operation), shows resorption of the medial border of the deciduous canine root, adjacent to the developing

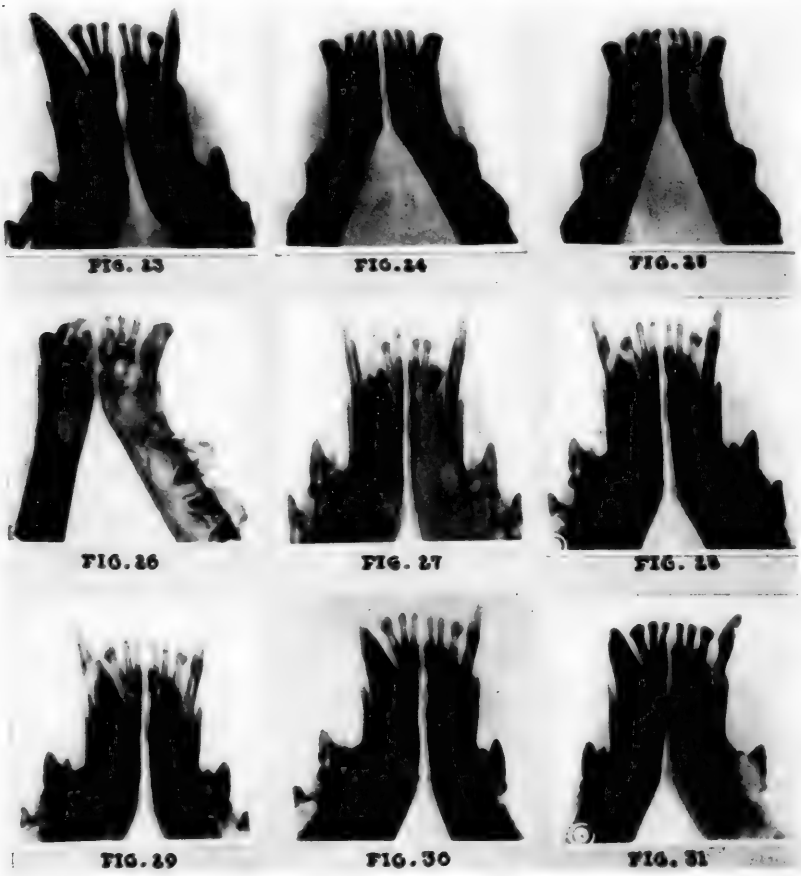


FIG. 23 FIG. 24 FIG. 25

FIG. 26 FIG. 27 FIG. 28

FIG. 29 FIG. 30 FIG. 31

germ of the permanent canine tooth on the control side. There is no indication at this time of resorption in the corresponding tooth on the operated side. Through the 74th day following operation, the radiographs show progressive resorption of the deciduous canine root on the control side (*figs. 28 and 29*). On the 75th day following operation, the deciduous canine tooth on the control side is shed (*fig. 30*). Meanwhile, (68 days after

operation), resorption of the root of the deciduous canine tooth on the operated side has begun (*fig. 29*). This resorption continues for 80 days following operation (*fig. 31*), and 5 days after the deciduous canine on the control side has been shed. Resorption of the deciduous canine on the operated side has progressed, and is comparable to the stage of resorption shown on the unoperated side in *fig. 29*. This tooth would have been shed in a few days.

Fig. 32 (cat #1) is a radiograph of the anterior part of the mandible from an adult cat in which a developing permanent canine tooth germ was removed at an early age and the animal allowed to develop. All the permanent incisors are present in their normal positions. The permanent canine is present on the left side but absent on the right.

FIG. 32
(CAT NO. 1)

CONCLUSIONS

(1) When the developing germ of the permanent canine tooth in a cat is removed, the deciduous canine tooth is shed.

(2) Resorption of the deciduous root on the operated side begins at a later time than that on the control side.

(3) There is some evidence in the radiographs of a slightly different mechanism of resorption when the pressure factor, due to the developing tooth germ, is eliminated. The nature of this mechanism is being sought in a histological study of the cases reported.

We are pleased to acknowledge the contribution to this work made by our technical assistant, Mr. Sidney Shapiro.

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EFFECTS OF DENTAL DETERGENTS ON CILIARY ACTIVITY[1]

R. L. PERRINE, A. H. THRONDSON, D.D.S., AND M. L. TAINTER, M.D.

Department of Physiology and Pharmacology, College of Physicians and Surgeons, A Dental School, San Francisco, Calif.

In the determination of actual or potential toxic effects of detergent materials, it is almost impossible to make clinical observations which are adequately controlled and completely reliable. Fortunately, animal tissues are available as a means for attacking the problem under more extreme conditions than could ever be attained clinically. When toxic effects can be demonstrated on animal tissues, they may be looked for with greater assurance in human tissues. Conversely, when toxic effects cannot be demonstrated experimentally, there is a greater assurance that such effects may not be expected from the ordinary clinical use of the agents concerned.

Since the time of Purkinje (1), ciliated epithelium has been a favorite means of detecting the presence of pharmacologic actions on the simplest type of motile protoplasm. The cilia are extrusions of epithelial cells, which beat more or less independently of central innervation and thereby give an external indication of the activity or vital functioning of the cells of origin. Therefore, they provide a very convenient means for observing the state of activity of the protoplasm of simple cells under whatever experimental condition may be imposed. Ciliary activity has been rather completely studied by many observers under a great variety of conditions. Those interested in the general responses of such ciliary preparations may refer to the excellent article of Lucas (2) and to the authors quoted in his report.

The effects of some of the agents used in dentifrices have been studied on cilia, by a number of observers, from standpoints other than their importance as detergents. For example, Kochmann (3) has reported that the volatile oils stimulate the ciliated epithelium in

[1] Supported in part by grants from the Research Commission of the American Dental Association, and the California State Dental Association.

concentrations within the therapeutic range. Lierle and Moore (4) found some depression with alcoholic solutions of menthol and thymol. However, the concentrations were higher than those obtainable in aqueous solutions. Similarly, Romanino (5) could demonstrate depressant effects with camphor compounds in oily solutions, which permitted higher concentrations than could be obtained in a watery solvent. A rather thorough discussion of the effects of the volatile oils and certain other compounds can be found in the doctorate thesis of Kühling (6), published in 1934. One other report of interest in the present connection is that of Maeda (7), who showed that boric acid and borates have definite inhibitory effects on tissue cultures, which are simple cell systems.

The agents used in the present study are common to a number of dentifrices on the market. The results, therefore, should be valuable in determining whether these dental products might produce undesirable effects on living tissues, when applied directly as are dentifrices. While the effects have obvious significance as to the responses of ciliated organs or structures, the results are to be interpreted, not solely as showing the actions of the agents on these, but, in addition, as indications of potential or actual effects, especially the undesirable or injurious, on functioning protoplasm in general.

METHODS

The method used was essentially that described by A. P. Richardson (8) in a recent publication. The ciliated epithelium of frogs was used, the esophagus from a frog being removed, cut longitudinally in half, and pinned on a board. The two strips were used simultaneously, one serving as a control, for comparison with the responses of the other, treated with the detergent. A fixed distance was measured off on the strips, and the time required for a small black mustard seed to be carried along this distance was measured with a stop watch. Five to ten alternate observations were made on each, and the averages taken as representing the normal or control time. Then one strip was irrigated with tap water and the other with tap water containing a given concentration of the agent in question. The series of observations on each were then repeated, and the speed on the medicated strip calculated as a percentage of the speed on the control strip. When there was a progressive change in the individual times observed, which would indicate that the full effects had not been obtained during the series of observations, the readings were continued until substantially constant values were obtained, and these were used for the calculation of the effect of the detergent. Some of the detergents were sufficiently insoluble and inert that they caused no demonstrable effects, even when applied in saturated solutions, with an excess of the powdered material in suspension. The soluble agents were applied in progressive dilutions, using a range of concentrations, from those without effect to those causing complete cessation of activity.

RESULTS

The results obtained are summarized in Table I, which shows also the number of frogs used for each concentration of each agent, and the average times observed. The insoluble materials, which showed no effect, are not included in Table I, but with these agents at least four frogs were used before it was decided that the effects were negligible or unimportant. It is seen that the most depressant agent tried was sodium alkyl sulfate, and that soap came next with about one-third

TABLE I

Comparative ciliary activity in various concentrations of detergents and aromatics used in dentifrices, expressed in percentage of control speeds

AGENT	AVERAGE NO. OF FROGS USED FOR EACH CONCENTRATION	CONCENTRATION OF DETERGENT OR AROMATIC IN PER CENT											
		0.000,01	0.000,1	0.001	0.002	0.01	0.1	0.25	0.5	1.0	2.0	4.0	10.0	20.0
Sodium Alkyl Sulfate	7.5		106	72	47	12	0	0	0	0				
Soap (U.S.P.)	5.0		108	105	81	45	9		0	0				
Soft Soap (U.S.P.)	7.1	104	106	103	99	96	54			0				
Ivory Soap	5.7		112	115	79	72	35			0				
Sodium Perborate	7.0	100	96	73		54	15			0				
Sodium Borate	6.2		93	85		69	18			0				
Boric Acid	4.1	96	100	114		108	100			102	96	46 (Sat)		
Sodium Acid Phosphate	5.5		97	100		87	93			92		58	48	0
Sodium Bicarbonate	6.0		96	93		89	72			51	0			
Alcohol	4.4		100	100		98	101			95	105	104	91	0
Glycerin	4.1			100		103	101			102	97	93	0	
Thymol	5.0		99	98		102	0 (Sat)							
Oil Cloves	2.5		100	101		96	0 (Sat)							
Oil Spearmint	3.0			104		105	0							
Oil Peppermint	4.0			102		97	35 (Sat)							
Oil Cinnamon	3.5		102	101		101	0							

less toxicity. This soap was the Castile soap of the United States Pharmacopeia. The official soft soap, which is more commonly used in paste or liquid preparations, was much less toxic. For sake of comparison, a commercial brand of soap was also included in this study, with the result that it was less depressing than the ordinary medicinal hard soap. Of rather high toxicity were the boric acid derivatives, sodium borate and sodium perborate, while boric acid itself was sur-

prisingly inert, even in concentrations of near saturation, that is, 4 per cent. Although sodium acid phosphate and sodium bicarbonate shift the hydrogen ion concentration far from the physiological limits for living protoplasm, these salts had little effect on ciliary motion, probably because their full effects cannot be exerted through the cell membrane. That is, the effects of inorganic salts and acids are largely exerted on the exterior, and not the interior, of cells. Alcohol also turned out to be relatively non-toxic, since there was very little depression of the cilia even with a 10 per cent concentration; 20 per cent was required for complete cessation of activity. Glycerin, which is used as a "smoothing" agent in toothpastes, had a toxicity only slightly greater than that of alcohol, since a 10 per cent solution was required for complete cessation of activity. From among the volatile oils, cloves, spearmint, peppermint and cinnamon behaved similarly in requiring a practically saturated solution in water, or about 0.1 per cent, to stop ciliary activity.

For sake of comparison, there has been calculated from these data the concentration of each agent required to produce a 50 per cent decrease in ciliary activity. This value has been taken for the comparison, since the concentration which causes a definite degree of effect can be determined more accurately than a concentration which produces no effect or a complete stoppage of activity. The values for 50 per cent reduction are shown in the first column of Table II. In order to translate these toxic concentrations into terms which would express relative toxicity, the values in Table II have been transposed by computing the reciprocals of the 50 per cent concentrations, shown in the second column, and arranging the agents in order of decreasing toxicity.

It can be seen that sodium alkyl sulfate was approximately 10 times, and soap 8 times, as depressant for the cilia as the nearest other agent, which was sodium perborate. However, because soap may be used in dentifrices in as high concentrations as 12.5 per cent (11), (Table II), whereas sodium alkyl sulfate is used in 1.25 per cent strength, the soap was about 8 times as irritating as the latter substance, when the quantities used are taken into consideration. Inasmuch as sodium perborate is used in from 10 to 100 per cent strengths in the mouth, its marked depressant action placed this salt at the head of the list of all

agents. This compound has very marked caustic actions which have been the source of much difficulty when it is used routinely in denti-

TABLE II

Relative toxicity for ciliated epithelium of detergents and aromatics in absolute concentrations and in proportion to the amounts used in dentifrices and other oral preparations[1]

COMPOUND	PERCENTAGE CONCENTRATION FOR 50 PER CENT DEPRESSION OF CILIA	RELATIVE TOXICITY	CONCENTRATION FOUND IN DENTIFRICES IN PER CENT	RATIO OF PERCENTAGE USED TO TOXIC CONCENTRATION
Sodium Alkyl Sulfate	0.00188	532	1.25	665
Soap (Hard, U.S.P.)	0.0025	400	12.5	5000
Sodium Perborate	0.0192	52.1	Up to 100	5206
Sodium Borate	0.0455	22.0	33	726
Oil Cloves	0.0533	18.8	1.5	28
Oil Cinnamon	0.0555	18.0	1.5	27.2
Thymol	0.056	17.9	1.5	26.8
Oil Spearmint	0.057	17.5	1.5	26.6
Ivory Soap	0.0635	15.7		
Oil Peppermint	0.0782	12.8	1.4	19.2
Soft Soap (U.S.P.)	0.167	6.0	10	59.9
Sodium bicarbonate	1.02	0.98	46	45
Sodium chloride	1.28	0.78	50	31
Sodium Chlorate	2.94	0.34	10	29.4
Potassium chlorate	3.00	0.33	10	30.0
Boric Acid	3.84	0.26	4	15.4
Glycerin	6.77	0.15	47	313
Sodium Acid Phosphate	8.8	0.11	10	90.9
Alcohol	14.51	0.068	10	147
Calcium Carbonate	No effect	0	94	0
Magnesium Carbonate—light	No effect	0	13	0
Sodium Metaphosphate	No effect	0	93	0
Dicalcium phosphate	No effect	0	58	0
Tricalcium phosphate	No effect	0	10	0
Benzoic acid	No effect	0	0.2	0
Camphor	No effect	0	1.5	0
Menthol	No effect	0	1.0	0
Oil Wintergreen	No effect	0	3	0

[1] The numerical values for Relative Toxicity are the reciprocals of the concentrations required to produce a 50 per cent depression of ciliary activity. The sodium chloride and sodium and potassium chlorate values are taken from A. P. Richardson (8). The compounds listed as having no effect were applied in saturated aqueous suspensions.

frices (11). There can be no question that its toxic effects on cilia reflect something of the undesirable local effects reported clinically.

Sodium borate was the only other compound in the series whose toxicity was great enough to make it comparable with these effective agents. Since borate may be used in dentifrices in concentrations as high as 33 per cent (11), its toxic effects were about the same as those of sodium alkyl sulfate, according to the results on cilia.

The volatile oils were in the upper part of the toxic range, as far as the absolute concentrations required for ciliary toxicity are concerned. These agents are used only in small concentrations as flavors, so that the possibility of producing toxic actions is very remote and probably nil. At the very bottom of the list of activity were the insoluble powders, namely, calcium carbonate, magnesium carbonate, sodium metaphosphate, and the 2 calcium phosphates. These materials are sufficiently insoluble that the only effect on the cilia was that of an inert powder. The same absence of action was observed with benzoic acid in saturated solution, and with the other flavors, namely, camphor, menthol, and wintergreen.

Included in Table II for sake of general interest are sodium chloride, sodium chlorate, and potassium chlorate, which were not investigated by us, but have been recently by A. P. Richardson (8), who used similar conditions. These salts have a very slight action on cilia. The chlorate-results are particularly significant, inasmuch as chlorate has been frequently criticized as a dentifrice constituent, largely because of possible systemic toxic effects. The fact that the chlorate has such a minor depressant action on the ciliated epithelium would indicate that this agent does not exert an oxidation on this tissue when directly applied. Therefore it follows that, since oxidation does not occur with chlorate, there can be no point in putting it into dentifrices or mouth washes, a conclusion which is in accord with recent conclusions of others.

DISCUSSION

The results of this study are part of a general investigation under way on the general actions on protoplasm of materials which enter into dentifrices, mouthwashes, and similar preparations. The present results in themselves are of little general significance if they are simply taken as indicating that a drug has or has not a toxic effect on cilia. When, however, it is realized that ciliary activity can be used to in-

dicate the presence or absence of a metabolic change in the protoplasm of the cell, the results can be interpreted in a somewhat broader sense. The observations leave no doubt that soap and sodium alkyl sulfate occupy a unique position insofar as effects on these sensitive indicators (cilia) are concerned. However, the observation that these two compounds are much alike, as far as the concentration required to depress the cilia is concerned, has added significance when it is remembered that the sodium alkyl sulfate has recently been introduced into several dentifrices. The object is to replace soap as a cleansing agent, and it is used in about one-tenth the concentration customarily employed for soap. A very interesting discussion of the chemical background for the sodium alkyl sulfate is given in a report of A. S. Richardson (9), and similarly valuable results, clinically and experimentally, have been published by Kitchin and Graham (10). The latter investigated the irritating properties of toothpastes containing this soap substitute in the clinic and in experimental animals, making a careful record of the cleansing properties, and of possible local irritant actions. No irritation or inflammation was observed in their study. This is in accord with the results of our study. In spite of the high absolute toxicity of this compound, the lower concentration in which it may be used would diminish its toxicity below that demonstrable in an ordinary period of observation. This does not answer the question as to whether the sodium alkyl sulfate may be suitable for use in dentifrices, when considered in the light of continued usage of these products. Since the possibility of chronic poisoning from prolonged usage has not yet been ruled out, the general use of sodium alkyl sulfate should await the results of such studies. Experiments by us are now in progress, and the results will be reported in due time. As for the other ingredients of the dentifrices, except the borate and perborate, it can be readily seen that the order of toxicity is so low in comparison to the soap and the sodium alkyl sulfate that even in spite of the greater concentrations of some of them, there is little chance of any undesirable local effects. This is in accord with clinical experiences which, however, have been obtained with a much less sensitive indicator, which may not be capable of detecting minor degrees of action.

An observation of some interest in other connections is shown in Table I, where it can be seen that some of the agents produced an

increased ciliary activity, that is, above 100 per cent, particularly when the agents were in dilute solutions. The function of cilia is, of course, to free a surface of particulate or other material by sweeping it toward the oral cavity. In some of these preparations, where an increased activity was demonstrated, the presence of insoluble particles or the concentration was such that a mild stimulation of the cilia resulted, with a corresponding increase in the speed of movement of the particle along the surface. This corresponds to the ordinary physiologic response of these cells to stimulation. But, the stimulation occurs apparently only when the proper range of concentration of the agent is used, and beyond the range the stimulation passes into a state of depression. This is consistent with pharmacological actions of drugs and other agents, in general.

CONCLUSIONS

1. The ability of various constituents used as detergents or aromatics in dentifrices has been investigated as to effects on ciliary activity under controlled conditions. Ciliated epithelium of the esophagus was employed for this purpose because the rate of ciliary activity is a sensitive index of protoplasmic depression or injury in epithelial surfaces.

2. It was observed that soap and sodium alkyl sulfate, a new detergent, had more than 10 times the toxic effect of other constituents, as judged by the concentrations required to depress ciliary movement. Also extremely toxic were sodium perborate and sodium borate, considering the concentrations which may be used in dentifrices.

3. The volatile oils and thymol, menthol, and camphor, used in saturated solutions, had practically no demonstrable effects on ciliary activity, so that the low concentrations used in dentifrices would be innocuous for living protoplasm. Similarly ineffective were boric acid, sodium bicarbonate, glycerin, alcohol, sodium acid phosphate, sodium chloride, sodium chlorate and potassium chlorate, considering the high concentrations necessary for effects.

4. Completely ineffective, and therefore non-toxic for cilia, were the insoluble detergent powders, namely, calcium and magnesium carbonates, sodium metaphosphate, and dicalcium and tricalcium phos-

phate, and benzoic acid, all of which were used in saturated solutions or suspensions.

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STUDIES IN TOOTH DEVELOPMENT

I. The 16 Microns Calcification Rhythm in the Enamel and Dentin from Fish to Man[1]

ISAAC SCHOUR, D.D.S., Ph.D., and MURRAY M. HOFFMAN, B.S., D.D.S.
Department of Histology, the University of Illinois College of Dentistry, Chicago, Illinois

Microscopic examination of ground and decalcified sections of enamel and dentin shows an incremental stratification of pairs of alternating light and dark layers. These layers follow a concentric pattern in transverse sections and resemble the annual rings in trees. The incremental stratifications have been more generally known as the striae of Retzius (enamel) and Owen's lines of contour (dentin). They are of physiologic significance because they reflect the nutritional and metabolic variations that occur during the growth and calcification of the dental tissues. Since no calcium withdrawal occurs in the teeth such records are permanent. The purpose of this study was to make a quantitative analysis of the incremental layers and to investigate their constancy and rhythmic nature in the teeth of various species.

REVIEW OF LITERATURE

Retzius (1837) first described the brownish parallel stripes in the enamel and Owen (1840) the corresponding lines of contour in the dentin. Owen speaks of contour lines that recur at intervals of 1/100 of an inch. v. Ebner (1922) speaks of lamellae that are sometimes only 18 to 20 μ apart. Andresen (1898) described cross striations in the dentin that were very regular. He reported no measurements. Asper (1916) found an average of 196 regular normal striae of Retzius in the crowns of ten human canines.

[1] This investigation has been aided by a grant from the Carnegie Corporation of New York and from the Graduate Research Board of the University of Illinois. Presented at the meeting of the International Association for Dental Research.

METHODS AND MATERIAL

Source of Material. The teeth represented various tooth types in normal animals of seventeen species from fish to man (Table I).[2]

The human teeth were selected at random from jaws obtained at autopsy and from extraction clinics and private dental offices.

Histologic Preparations. Decalcified sections were stained as a rule with hematoxylin and eosin. Most of the ground sections were prepared by hand with carborundum and arkansas stones.

Method of Measuring. The measurements were made from the beginning of one dark band to the beginning of the next dark band along the enamel rods or dentinal tubules since they mark the path of growth. An effort was made to avoid tangential fields. A micrometer eyepiece which was graduated to read up to one-tenth of one micron was used. Five thousand, four hundred and sixty-six measurements were made on the incremental pairs of dark and light layers in the enamel and dentin of 183 teeth of 17 species (Table I, *Text fig.* 1). Eighteen hundred of these were made from human teeth.

DESCRIPTIONS OF PHOTOMICROGRAPHS

PLATE I

1. Longitudinal section of the dentin of the *Leuciscus rutilus* showing the normal 16 μ stratification (from Weidenreich, 1929) $\times$ 140.

2. Decalcified section showing the incremental stratification of the light and dark bands in the dentin of the incisor of a normal rat. The average of the measurements is 15.91 μ. $\times$ 270.

3. Decalcified section of the incremental stratification in the dentin of the incisor of a normal ground squirrel. The average of the measurements is 16.11 μ. $\times$ 180.

4. Longitudinal decalcified section of the dentin of the incisor of a rabbit illustrating normal increments. The average of the measurements is 16.05 μ. $\times$ 350.

5. Longitudinal ground section of the molar of an opossum illustrating the incremental bands in enamel. Average width of incremental pairs is 16.20 μ. $\times$ 285.

6. Dentin of the same section as figure 5. Average width of pairs of light and dark layers is 15.62 μ. $\times$ 285.

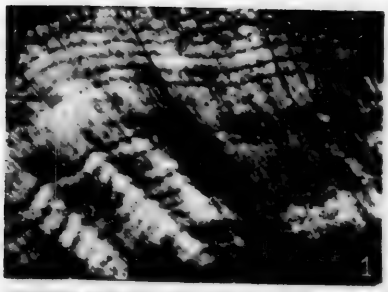
The statistical method of Sinnott and Dunn (1925) was used to compute and interpret our results. Measurements in the enamel were made only in ground sections since the enamel is lost in decalcified sections. Measurements in the dentin were made in both ground and decalcified sections.

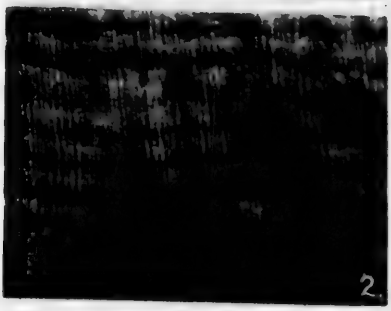
In addition, over 200 measurements were made of the incremental zones in the dentin of incisors of animals that were subjected to severe disturbances in calcium metabolism (gonadectomy in the ground squirrel, Schour (1936a,) Parathyroidectomy in rat, Schour, Chandler and Tweedy (1937)).

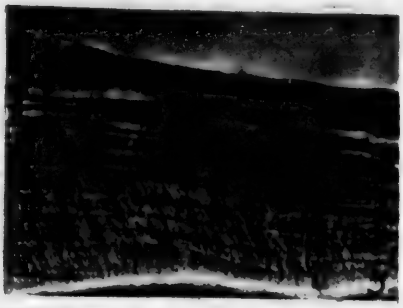
FINDINGS

Description of the Incremental Pairs of Light and Dark Layers. The appearance of the light or dark components of each increment is

[2] The material from fishes, amphibians and reptiles was obtained through the courtesy of Dr. R. Kronfeld.







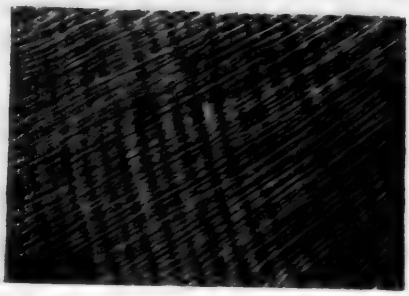


PLATE I

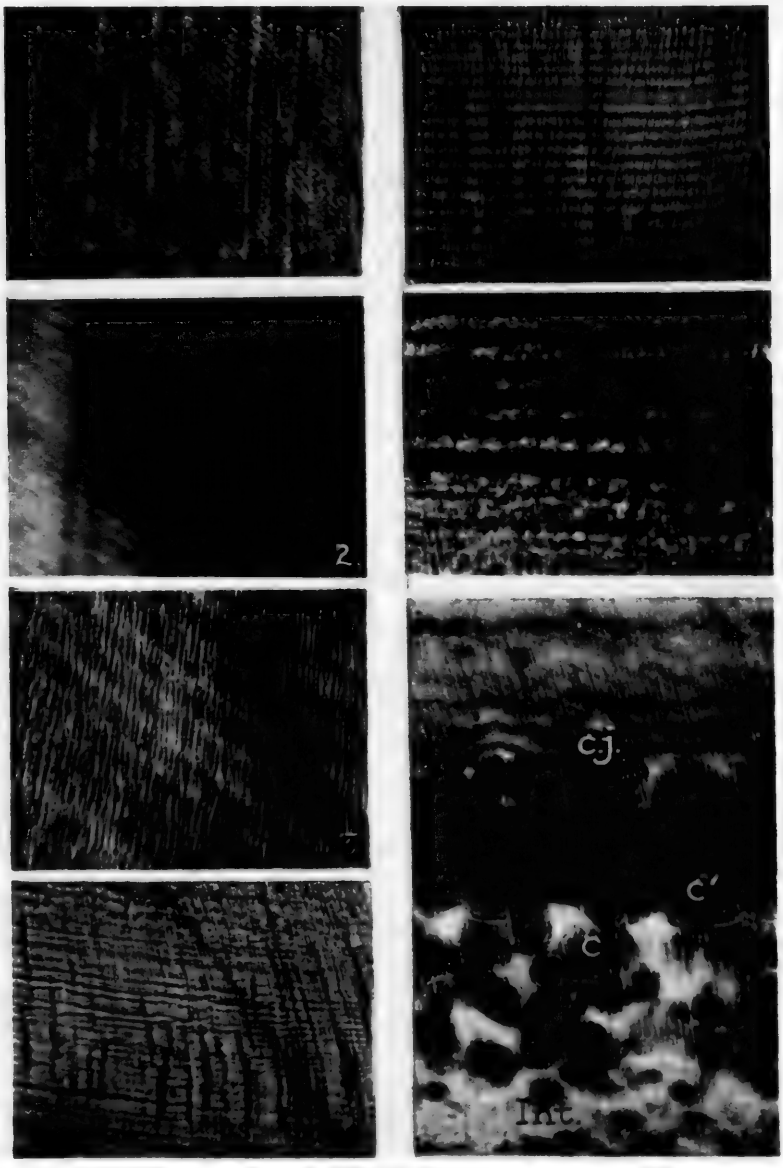


PLATE II

dependent upon the type of illumination and the variables encountered during the preparation (*Plate I, 1–6; Plate II, 3–6*).

In the enamel, under high power, the increments appear as series of steps consisting of particular portions of interprismatic substance and cross striations of successive enamel rods (*Plate II, 1*). In the human enamel, in particular, the height of each vertical step equals

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PLATE II

1. Ground section of the enamel of the incisor of a pig illustrating the normal incremental light and dark bands of calcification. Measurements were made along a path parallel to the enamel rods. Average of the measurements is 15.58 μ. × 350.

2. Decalcified section of the enamel matrix of a molar of a sheep illustrating normal increments of calcification. Note that the dark band is composed of two peripheral dark stripes and one intervening light stripe. In measuring the incremental pairs of light and dark bands it was necessary to begin at any one of the peripheral dark stripes of one dark band and end at a corresponding dark stripe of next dark band, following a path which is parallel to the enamel rods. The average of the measurements was 16.39 μ. × 700.

3. Decalcified section of the normal incremental stratification of the dentin in a cat. Average of measurements equals 15.8 μ. × 350.

4. Ground section of the dentin of a canine of a dog showing the normal incremental stratification which recurs at an average interval of 15.8 μ. Note some of the accentuations which recur at intervals of multiples of 16 μ. × 135.

5. Decalcified section of the normal dentin of a molar of Macacus rhesus monkey, illustrating the rhythmic incremental stratification recurring at an average interval of 16.53 μ. × 192.

6. Transverse ground section of human enamel in the gingival third of the crown, showing normal incremental bands (striae of Retzius). Average measurements per pair of light and dark bands for this field is 16.29 μ. Note the direction of incremental bands in relation to enamel rods. × 270.

7. Decalcified section of the dentin in the incisor of a rat placed on a rachitic diet. C, early calcospherites which are forming; c′, calcospherites which are joining and are beginning to give the incremental stratification; c.j., completely joined calcospherites producing the completed incremental stratification. Int., interglobular dentin characteristic for rickets. × 380.

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one enamel globule, the width of each step equals that of an enamel rod. The layers course at an oblique angle to the enamel rods and take a position which is identical with the surfaces of the enamel that obtain during the successive stages of enamel apposition.

In decalcified sections of dentin, the more calcified layer of each incremental pair stains predominantly with hematoxylin. The less calcified component shows a globular texture and stains more readily

TABLE I

Source, number and measurements of the incremental stratification of pairs of light and dark layers in the enamel and dentin

SPECIES	SOURCE			NUMBER OF TEETH	NUMBER OF MEASUREMENTS[1]	MEAN[2]	STANDARD DEVIATION FROM MEAN	MEAN ERROR[3]
	Tooth type		Tissue					
	Continuous growth	Limited growth						
Fish[4]			Dentin	11	213	15.97	±1.56	.186
Crocodile			Dentin	1	48	15.91	±1.09	.272
Opossum		C	Dentin	1	41	15.78	±2.82	.443
		M	Enamel	1	14	15.96	±.611	.163
Mastodon		M	Enamel	1	31	16.05	±2.31	.417
		M	Dentin	1	16	16.16	±.73	.182
Giant beaver	I		Enamel	1	21	15.79	±2.14	.475
			Dentin	1	51	16.21	±1.14	.163
Rat	I	M	Enamel	14	212	16.04	±1.60	.219
	I	M	Dentin	38	786	15.89	±3.51	.217
Ground squirrel			Enamel	1	21	15.72	±2.01	.42
	I		Dentin	3	150	15.38	±2.54	.36
Guinea pig	I		Dentin	4	171	15.64	±1.57	.223
Gray squirrel		M	Dentin	2	102	15.78	±1.15	.197
Rabbit	I M		Dentin	7	471	15.86	±3.09	.374
Cat		I C M	Enamel	1	15	15.87	±2.50	.266
			Dentin	3	96	15.91	±1.62	.279
Dog		C	Enamel	2	84	15.25	±2.045	.393
		C	Dentin	4	231	15.84	±2.49	.270
Pig			Enamel	2	42	15.77	±2.07	.227
		M	Dentin	6	101	15.73	±2.19	.216
Sheep		M	Dentin	2	39	15.92	±.645	.178
			Enamel	1	11	16.39	±1.21	.172
Cow			Enamel	1	60	15.72	±2.08	.465
		M	Dentin	1	144	15.89	±2.11	.323
Macacus rhesus		I	Enamel	5	63	16.00	±.893	.193
		I	Dentin	11	432	15.85	±2.325	.2004
Human		I. C. P. M.	Enamel	34	465	15.87	±2.87	.230
		I. C. P. M.	Dentin	51	1335	15.85	±4.36	.208
Total			Enamel	50	1039			
			Dentin	133	4427			
Sum Total				183	5466			

[1] Each measurement was arrived at by taking three trials and recording the mean.

[2] Mean = a ± (c × i) where a = assumed mean
c = sum of number of measurements from assumed mean divided by number of measurements
i = incident

[3] Mean Error = $\frac{\text{standard deviation } (\sigma)}{\text{number of measurements}}$

[4] Fish = Pike
Shark
Angle fish (Loplius piscatorus)

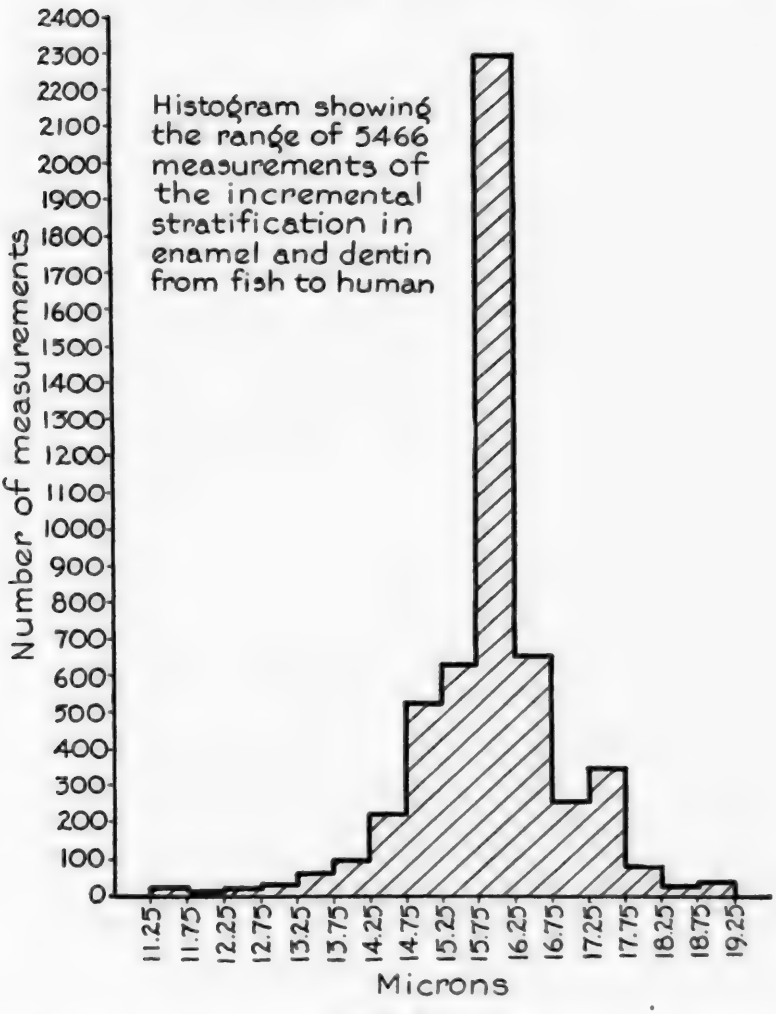

FIGURE 1

with eosin. The layers run at nearly right angles to the dentinal tubules at any given point and mark the various pulpal surfaces that obtain during the successive stages of dentin apposition.

Findings by Micrometer Measurements. Table I gives the source,

number and measurements of the pairs of light and dark layers in the enamel and dentin. The measurements in the dentin were not significantly different in ground and decalcified sections. The mean value (15.92 μ) per pair of light and dark layers approximated 16 μ with a very small probable error. Eighty per cent of the measurements range between 15 and 17 microns; 66 per cent of the measurements range between 15.5 and 16.5 μ. The range of measurements seen in *fig. 1* shows extremes of 11.2 and 19.2 μ and a sharp elevation at about 16 μ. There was no significant difference in the width of the incremental pairs between enamel and dentin, between teeth of continuous or limited growth, between incisors, canines or molars, and between the teeth of the various species studied so far.

Measurements made from photomicrographs used in texts and reports where the exact magnification was given [Asper (1916), Hopewell-Smith (1918), Mummery (1924), Walkhoff (1924), Weidenreich (1929)] are included in Table I and were not significantly different from those obtained in our sections (Table I, *Plate I, 1*).

Measurements Showing Multiples of 16 Microns. Accentuated stratifications are often seen in human teeth particularly in the enamel. One hundred and fifty measurements made in the enamel of 8 human anterior and posterior teeth picked at random revealed prominent striae of Retzius. The intervals between these successive layers ranged in multiples of 16, from 32 μ to 128 μ. Closer examination disclosed that these broader zones were made up of separate units of the 16 μ layers and were thus superposed upon the basic 16 μ incremental stratification. Corresponding Owen's lines of contour were less common. In many human teeth the striae of Retzius were very prominent in the gingival enamel and recurred at intervals of 32 microns. This stratification in multiples of 16 μ is also found in normal and experimental laboratory animals.

DISCUSSION

The Occurrence of Rhythmic Stratification in Plant and Animal Life. McGuigan and Brough (1923) have pointed out that rhythmic banding is a common phenomenon in biology, and have given as examples the lamellae of the Haversian canals in bone; the pigmentation of the iris, the color of butterflies' wings, the annual growth rings of trees, the

shells of mollusca, and the bands of starch grains and of many seeds. We can readily add the 16 μ incremental rings of the tooth.

The 16 μ Calcification Increment as a Constant Biologic Unit of Calcification. The finding that enamel and dentin possess the same quantitative rhythmicity in calcification is surprising in view of the fact that enamel and dentin are different not only in the degree of their calcification but also in respect to origin, formation, and structure. The constant and widespread recurrence of the 16 μ rhythm throughout the numerous species that have been examined from fish to man in teeth of different types; deciduous and permanent, of continuous and limited growth, incisors, canines, premolars and molars, is notable. All these facts suggest a common physiologic and physico-chemical basis for the 16 μ rhythmicity in the calcification processes of both the enamel and dentin. The 16 μ incremental rhythm may thus be regarded as a constant and common biologic unit of calcification of enamel and dentin, and the 16 μ incremental rings constitute the common denominator for the calcification of both enamel and dentin.

The Possible Physico-Chemical Basis for the 16 μ Calcification Rhythm. Enamel and dentin calcification is a process of precipitation of inorganic calcium salts into a colloidal organic matrix. Andrews (1919) points out that each enamel globule represents a calcospherite formation. Mummery (1924) states, "The stratification of the dentin is due to the structure of the calcospherites and is the result of the rhythmic or periodic deposit of the lime salts in a colloid."

The calcospherites are demonstrated beautifully in the dentin of rachitic rats (*Plate II*, 7), where they are isolated and therefore conspicuous. Normally, the individual calcospherites would fuse into a homogenous layer of calcified dentin. In rickets the dentin is deprived of the precipitating calcium and the calcospherites are insufficient in number to fuse into a whole homogeneous mass (*Plate II*, 7).

We may visualize the calcification process in terms of the rhythmic aggregation of calcospherites which fuse more or less completely and give rise to successive layers. These assume an incremental arrangement in conformance with the outline of the pulp or the surface of the enamel, just as Liesegang rings are deposited according to the form of the vessel.

The arrangement of the calcification pattern in intervals of 16 μ in

both the enamel and dentin may be the specific response that is characteristic for the precipitating subsances (calcium salts) and the organic matrix involved. According to Thompson (1917), Liesegang rings arrange themselves in a definite pattern, depending upon the nature of the substance formed, and the medium in which it is formed.

A physiological rhythmic deposition or secretion of the mineral solution possibly associated with other basic rhythms or with alternate rhythms, cellular activity and exhaustion, would account for an alternately more complete and less complete fusion of calcospherites. The more complete fusion of the latter would give rise to the better calcified layer, the less complete fusion to the less calcified layer.

The Striae of Retzius and Owen's Lines of Contour as Accentuated Incremental Rings. The pairs of light and dark layers which recur at intervals of 16 μ are incremental in pattern and reflect a basic physiologic rhythm in calcification. As a result of systemic disturbances affecting calcium metabolism the incremental ring forming at any given time may become accentuated. These accentuations have been described as the striae of Retzius and Owen's lines of contour. The histologic appearance of the 16 μ incremental rings can be used as an index of the degree of calcification. Depending on the degree of the disturbance in calcification, a given stria of Retzius or Owen's line of contour may involve only the less calcified component of the normal incremental ring, an entire 16 μ increment, or several incremental rings. It is very interesting that the intervals between various accentuations tend to recur in multiples of 16 from 32 μ to 128 μ.

The kymographic accuracy with which the enamel and dentin in their formative and calcifying periods register many of the physiologic and systemic experiences of the individual constitutes the basis for tooth ring analysis as an aid in the assessment of health and disease (Schour, 1937). The accentuations of the normal increments may be produced by constitutional or environmental factors such as birth (neonatal ring, Schour, 1936), or by various pathologic conditions that disturb calcium metabolism (Schour, 1938). Experimental accentuations of the normal 16 μ rings can readily be produced by various endocrine and dietary disturbances or by injections of sodium fluoride.

In experimental conditions in which calcification is stopped, as in the wide dentinoid zones in rickets, the incremental stratification is

absent. In experimental conditions where the calcification process is disturbed but not stopped (parathyroidectomy or fluorosis), there is an accentuated staining of the normal incremental stratification, but no change in the 16 μ rhythm. This quantitative factor is not modified even in the incisors of rats subjected to hypophysectomy or vitamin A deficiency.

Animals which were born and reared in the laboratory under normal conditions showed less frequent accentuations than those that were caught in the field (ground squirrels). The latter are subject to greater imbalances in calcium intake and calcium metabolism than are the carefully controlled laboratory animals which were kept under more favorable and constant conditions in respect to diet and temperature. A similar reaction can be observed in the teeth of human children. Striae of Retzius and Owen's lines of contour are more frequent in the teeth extracted in free dental clinics than in the teeth obtained from physicians and dentists whose patients had more favorable environmental conditions.

General considerations. The wide distribution and striking constancy of the 16 μ incremental rhythm suggests a promising field of investigation on the calcification rhythm in various normal and pathologic accretions of nondental nature. The time factor of the 16 μ increment was investigated in various species and the findings are presented in a separate report.

SUMMARY AND CONCLUSIONS

The width of the pairs of light and dark incremental layers that constitute the normal stratification of enamel and dentin was measured in various tooth types in 17 species from fish to man. A total of 5,466 micrometer measurements were made. The mean value per pair of light and dark layers, for the entire group of teeth approximated 16 microns with a small deviation and probable error; 80 per cent of the measurements fell between 15 and 17 microns; 66 per cent between 15.5 and 16.5 microns.

There was no significant difference in the width of the incremental layers between the enamel and dentin; between the teeth of various species; between teeth of limited or continuous growth; or between incisors, cuspids or molars.

The following conclusions may be drawn from the data obtained:

1. The enamel and dentin of all the species studied show a common basic incremental calcification rhythm which recurs at intervals of 16 μ.

2. The common basis of this 16 μ calcification unit is possibly associated with the physico-chemical factors concerned in calcospherite formation and the Liesegang ring phenomenon.

3. Striae of Retzius and Owen's lines of contour constitute physiologic or pathologic accentuations of the normal incremental rings and tend to recur at intervals which are multiples of 16 μ.

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